VOLUME 38 NUMBER 1

# ARCHIVES OF PATHOLOGY



JULY 1944

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO 10, ILLINOIS. ANNUAL SUBSCRIPTION, $6.00

Entered as Second Class Matter Jan. 23, 1926, at the Postoffice at Chicago, Under the Act of Congress of March 3, 1879

## CONTENTS OF PREVIOUS NUMBER

### JUNE 1944, NUMBER 6


The Adrenal Medulla in Various Diseases: A Histophysiologic Study. Major Ralph L. Drake, Medical Corps, Army of the United States; Lieutenant Commander James S. Hibbard (MC), U.S.N.R., and C. Alexander Hellwig, M.D., Wichita, Kan.

Encephalitis Complicating Virus Pneumonia: Report of a Case with Autopsy. Helen Ingleby, M.D., Philadelphia.

Anomalous Pulmonary Veins. Charles W. Hughes, M.D., and Peter C. Rumore, M.D., Chicago.

Primary Neoplasms of the Liver. Wesley N. Wagel, M.D., Cincinnati.

Feather Germ Reaction to Urine from Patients with Cancer and Other Conditions: A Preliminary Study. Mary Jahn, Ph.D., College Park, Md.

Case Reports:

A Mixed Tumor of the Salivary Gland Type on the Left Hand. Benjamin Highman, M.D., Bethesda, Md.

Myasthenia Gravis: Report of a Case with Autopsy. Fred S. Fiesen, M.D., and Sanford Goodman, M.D., Cleveland.

Laboratory Methods and Technical Notes:

Myelin Staining by a Fixed Schedule for the Occasional User. R. D. Lillie, M.D., Bethesda, Md.

Historical Review:

Origins of the Cell Concept in Pathology. Captain Hans G. Schlumberger, Medical Corps, Army of the United States.

Books Received.

General Index.

ARCHIVES OF PATHOLOGY

VOLUME 38 JULY 1944 NUMBER 1



# INFANTILE TOXOPLASMOSIS

## WITH A REPORT OF THREE NEW CASES, INCLUDING TWO IN WHICH THE PATIENTS WERE IDENTICAL TWINS

WOLF W. ZUELZER, M.D.

DETROIT

Toxoplasma, a protozoan parasite of wide distribution in animals, recently has been found to be pathogenic for man. Four main types of human toxoplasma infection are now recognized:

1. A congenital type, with onset in utero, manifesting itself chiefly as fetal or neonatal encephalomyelitis, often fatal within the first few weeks of life but sometimes asymptomatic until later infancy or early childhood, when the presence of residual lesions becomes apparent.

2. An acquired encephalitic type in older children.

3. An acute febrile type resembling typhus or spotted fever, occurring in adults.

4. A latent infection recognized only by the presence of neutralizing antibodies in the serum, occurring in adults (and probably also in children).

The morphologic characteristics of toxoplasma are well established.[1] When examined in air-dried films, the parasites have a rounded, piriform or crescentic shape and measure from 4 to 7 microns in length and from 2 to 4 microns in width; in sections of fixed tissues they may be considerably smaller. They have a distinct nuclear chromatin mass and a cytoplasm which appears clear and homogeneous in most stains. They appear to divide by binary fission and, according to some authors, notably Sabin and Olitsky,[2] are obligate intracellular parasites.

Wolf, Cowen and Paige[3] were the first to prove the occurrence of toxoplasmosis in man when they identified the parasites in a fatal case of infantile encephalomyelitis on the basis of morphologic characteristics, successful transmission to animals and demonstration of immunity against the human strain in rabbits and mice immune to a known strain of toxoplasma. They recognized as further instances of infantile toxoplasmosis a case of granulomatous encephalitis previously reported by them,[4] and 3 cases which they found recorded in the literature.[5] Subsequently Paige, Cowen and Wolf[6] reported 3 new fatal cases together with a case already published but not recognized as one of toxoplasmosis by de Lange.[7] Pinkerton and Weinman[8] studied the tissues of a premature infant in a case which had been reported originally by Hertig[9] as one of sarcosporidiosis and identified the parasites as toxoplasma on morphologic grounds. A further example of infantile toxoplasmosis was recently reported by Steiner and Kaump.[10] These authors also briefly mentioned a second case, referred to them by Sailer. They were able to study a section of the brain and arrive at the diagnosis of toxoplasmic encephalitis. Thus, in all, 12 pathologically verified cases of infantile toxoplasmosis are recorded in the literature. A complete autopsy was performed in 9 of these cases.

The lesions in the central nervous system as described by Wolf, Cowen and Paige[11] consisted of disseminated areas of necrosis with a tendency to calcify, miliary granulomas and hydrocephalus. In all cases in which an examination was made, focal chorioretinitis was found.

From the Department of Pathology, Children's Hospital of Michigan, and the Department of Pathology, Wayne University College of Medicine.

1. Sabin, A. B.: Toxoplasmosis, in De Sanctis, A. G.: Advances in Pediatrics, New York, Interscience Publishers, Inc., 1942.

2. Sabin, A. B., and Olitsky, P. K.: Science **85**: 336, 1937.

3. Wolf, A.; Cowen, D., and Paige, B. H.: Am. J. Path. **15**:657, 1939.

4. Wolf, A., and Cowen, D.: Bull. Neurol. Inst. New York **6**:306, 1937.

5. (*a*) Jankû, J.: Časop. lék. česk. **62**:1021, 1054, 1081, 1111 and 1138, 1923. (*b*) Torres, M. C.: Compt. rend. Soc. de biol. **97**:1778, 1928. (*c*) Richter, R.: Arch. Neurol. & Psychiat. **36**:1085, 1936.

6. Paige, B. H.; Cowen, D., and Wolf, A.: Am. J. Dis. Child. **63**:474, 1942.

7. de Lange, C.: Ztschr. f. d. ges. Neurol. u. Psychiat. **120**:433, 1929.

8. Pinkerton, H., and Weinman, D.: Arch. Path. **30**:374, 1940.

9. Hertig, A. T.: Am. J. Path. **10**:413, 1934.

10. Steiner, G., and Kaump, D. H.: J. Neuropath. & Exper. Neurol. **3**:36, 1944.

11. Wolf, Cowen and Paige.[3] Wolf and Cowen.[4] Paige, Cowen and Wolf.[6]

Occasionally lesions were encountered in other organs, notably in the myocardium.

In a subsequent article Cowen, Wolf and Paige [12] reported 6 cases in which the patients survived beyond infancy, and established the clinical picture of infantile toxoplasmosis. According to these authors, the main features of the early phase are convulsions and other neurologic symptoms, internal hydrocephalus, bilateral chorioretinitis and the presence of foci of intracerebral calcification. Occasionally prolonged jaundice and enlargement of the liver and the spleen are observed. The spinal fluid is xanthochromic, and its cell count and protein content are increased. In patients surviving the early stages, the hydrocephalus persists or increases, the chorioretinitis heals but causes permanent impairment of vision and searching nystagmus, and mental development is apt to be retarded.

In 2 older children with acquired toxoplasmosis Sabin [13] observed a different picture, characterized by acute encephalitic symptoms. One of these patients died, and scanty lesions of miliary granuloma were found in the brain.

The influence of age on the manifestations of human toxoplasmosis was even more strikingly brought out by the description by Pinkerton and Weinman [8] and Pinkerton and Henderson [14] of the changes in 3 adults who died of the disease. In these patients the clinical picture was that of an acute exanthematic disease. The lesions observed at autopsy were located predominantly in extraneural tissues. Outstanding was diffuse interstitial pneumonitis, but lesions and parasites were found in the spleen, the liver and other organs and consisted of foci of necrosis and inflammatory changes.

An important step in the study of the disease was taken by Sabin and Olitsky [2] and Sabin,[15] who developed a method of demonstrating neutralizing antibodies against Toxoplasma in the serum. Sabin was able to demonstrate such antibodies not only in the serum of patients with toxoplasmosis but in that of their mothers and sometimes in that of other members of the family as well.[1] In his monograph he briefly summarized 9 cases of toxoplasmosis in infants and children in which he confirmed the clinical diagnosis by this immunologic method. Using a modification of Sabin's method, Cowen, Wolf and Paige [12] examined the maternal serum in 8 cases of infantile toxoplasmosis and demonstrated antibodies against Toxoplasma in 5 of these. These observations furnished additional evidence in favor of the assumption, made from the first, that in at least some cases of infantile toxoplasmosis the infection begins in utero, and at the same time indicated the existence, perhaps widespread, of silent toxoplasmic infection in man. At present the mode of transmission to human beings is not clearly understood.

The total number of acceptable cases of human toxoplasmosis (counting Sabin's briefly summarized cases) now stands at 32. To these, 3 new cases are added in this report, with complete autopsy reports on 2. The third patient is alive and has been under close observation during the period from birth to the present age of 7 months. His case is included because he is the identical twin brother of one of the patients on whom autopsy was performed.

## REPORT OF CASES

CASE 1.—R. S., a white boy 9 days old, was admitted to the Children's Hospital of Michigan, Sept. 7, 1943 with the chief complaints of lack of appetite, drowsiness and convulsions.

The patient was born at home approximately two weeks after the calculated term. Labor had been rapid and spontaneous. The delivery was of the normal, cephalic type. The membranes were ruptured by the attending physician just before the child was born. The patient cried immediately but shortly after birth had a cyanotic spell. On the third day another episode of cyanosis occurred during a bath. The respirations were always rapid and "panting" in character. The infant seemed apathetic and nursed poorly. At the age of 8 days he refused the breast, and the mother noted twitching movements of his face, hands and, a little later, his legs. At first these attacks lasted only a few minutes but recurred frequently, and soon the child was in a state of continuous generalized convulsions with opisthotonos. The temperature rose to 101 F. The patient was brought to the hospital on the following day.

*Family History.*—The family had lived in a small town in Michigan until November 1942, when they moved to Detroit. The father, 28 years of age, worked in an aircraft factory and was in good health. The mother, 24 years old, had been complaining of nervousness and weakness for the last two years but was otherwise well. There were 2 older children, aged 6 and 3 years, both said to be healthy. The family lived in a framework house, which during the mother's last pregnancy had been temporarily infested by rats. There were no pet animals in the house. The mother recalled that her family had kept a dog when she was a child.

*Prenatal History.*—The mother's two preceding pregnancies had been normal. There had been no miscarriages. No blood tests had ever been made. During the last pregnancy the mother had noted that the fetus was unusually quiet and, as she described it, had frequently "doubled-up" and maintained this position for hours at a time but had almost never moved or kicked. During the last month the mother had experienced considerable backache.

*Physical Examination.*—The infant was newborn, well developed and well nourished. The skin was slightly icteric. There was no cyanosis. The head was arched

12. Cowen, D.; Wolf, A., and Paige, B. H.: Arch. Neurol. & Psychiat. **48**:689, 1942.

13. Sabin, A. B.: J. A. M. A. **116**:801, 1941.

14. Pinkerton, H., and Henderson, R. G.: J. A. M. A. **116**:807, 1941.

15. Sabin, A. B.: Proc. Soc. Exper. Biol. & Med. **41**:75, 1939; footnotes 1 and 13.

backward. Clonic convulsive movements of the arms and twitchings of the right eye were noted. The remainder of the findings were noncontributory. The ocular fundi were not examined. The temperature was 99 F.

*Laboratory Data.*—The hemoglobin content of the blood was 24.1 Gm. per hundred cubic centimeters. The white blood cell count was 21,200 per cubic millimeter. The differential count was polymorphonuclear leukocytes 6 per cent, lymphocytes 92 per cent, monocytes 1 per cent and eosinophils 1 per cent. The Kline test was negative. Culture of blood taken on admission yielded no growth for five days. A sample of the urine was not obtained.

The spinal fluid seemed to be under normal pressure. It was markedly xanthochromic. The cell count was 17 per cubic millimeter (90 per cent polymorphonuclear leukocytes, 10 per cent "mononuclear cells"). The Pandy test showed globulin in the fluid (3 plus). Sugar was present in normal amounts, according to a crude quantitative test. An examination for parasites was not made.

*Roentgen Examination.*—Only the chest was examined. Both lungs appeared well aerated. The bronchovascular markings on both sides were increased. The heart was slightly bulbous in shape but was not considered definitely abnormal.

*Course.*—The infant was given a subcutaneous infusion of saline solution and sodium sulfadiazine. He received 1 ampule of synkamin (a preparation of vitamin K—5,4-amino-2-methyl-1-naphthol). He appeared somewhat stuporous but at times cried vigorously. The opisthotonos persisted, and cyanosis of the hands and feet was observed. On the second hospital day intense cyanosis of the entire body developed and persisted although the patient was placed in an oxygen tent. The temperature of the skin dropped markedly, and external heat was applied. The child was weak and unable to take feedings. On the third hospital day the respirations became labored and noisy, the cyanosis increased and the patient died at the age of 11 days.

*Gross Observations at Autopsy.*—A complete autopsy was made four and a half hours after death. The body measured 50.5 cm. There was cyanosis of the lips and gums. A thin yellow liquid oozed from the nostrils. The right palpebral fissure was narrow, and the eye barely could be visualized. The left eye appeared normal in size. The conjunctivas were injected. The left pupil was round and regular. The abdomen was mildly distended. The umbilical cord was black and dry. A small amount of crusted yellow exudate covered its base. The intraperitoneal portions of the umbilical vessels appeared normal.

The heart was slightly large, globular in shape and weighed 28 Gm. The myocardium was of a peculiar pale pinkish yellow color, fairly firm, and of uniform appearance on the cut surface. The valves appeared normal.

The lungs were slightly heavy and rubbery, especially in the posterior portions, and the cut surfaces were meaty and congested in appearance.

The spleen was enlarged and weighed 18 Gm. It was dark, purple and firm and had indistinct follicular markings on the cut surface. A small accessory spleen had similar characteristics.

The liver weighed only 90 Gm. It did not appear unusual. The remaining organs except for the brain appeared grossly normal.

The brain weighed 350 Gm. There were no subdural or subarachnoid hemorrhages. The hemispheres were well formed, and no exudate was noted on gross examination. On coronal sections made after fixation in formaldehyde, numerous opaque, yellowish white areas were noted in the hemispheres. These lesions were especially numerous in the band of cortical tissue, particularly at the bottom of the cerebral sulci, but were also noted in the white matter and in the basal ganglions. No gross lesions were found in the cerebellum, the pons varolii or the medulla oblongata. The ventricles were symmetric and lined by smooth, translucent ependyma; there was no dilatation or other evidence of obstruction. The choroid plexuses appeared delicate.

*Microscopic Observations.*—The tissues were fixed in Zenker's fluid except for the brain and spinal cord, which were fixed in toto in a 4 per cent solution of formaldehyde. Sections were cut at 4 to 6 microns and stained with hematoxylin-eosin, Giemsa's stain, phloxine–methylene blue, Heidenhein's iron-hematoxylin, Goodpasture's stain, Ziehl-Neelsen's carbol-fuchsin, Mallory's aniline blue and Mallory's phosphotungstic acid–hematoxylin.

(*a*) Heart: There was widespread infiltration of the myocardial fibers and of the interstitium with inflammatory cells (fig. 1 *A*). These consisted of mononuclear cells, plasma cells and a few eosinophilic leukocytes. The infiltrations, although often poorly defined, were mainly focal. In most of the foci the myocardial fibers had undergone hyaline necrosis and fragmentation. The process was slightly more marked in the inner layers of the myocardium than elsewhere. Many fairly well preserved fibers contained collections of parasites of characteristic appearance (fig. 1 *B*). The individual parasites were small ovoid or rounded bodies, measuring on the average 3 to 4 by 2 to 3 microns in paraffin sections of material fixed in Zenker's fluid. In each parasite a round chromatin mass, usually placed near the thicker end of the structure, was distinguishable from an eosinophilic cell body, in which now and then minute basophilic particles could be made out. The nucleus was sometimes surrounded by a faint halo. The parasites occurred in compact aggregates, which appeared to lie in clear spaces within the myocardial fibers, usually close to nuclei. On the average 8 to 10 parasites were present in a given compartment, but as many as 60 were counted in 1 instance. A few fibers contained only 2 or 3 parasites, about which a definite space seemed to be lacking. A distinct membrane was not demonstrable even around the largest collection of parasites. The parasitized fibers usually were slightly swollen and had lost their transverse striation in the portions close to the parasites but were otherwise intact. Necrosis or inflammatory changes were almost never encountered in the vicinity of parasitized fibers.

Single parasites were often found in areas of beginning necrosis and peripherally in larger areas of necrosis, sometimes in considerable numbers. However, they were almost never found in the centers of foci of extensive necrosis and cellular infiltration. Some of the myocardial fibers in otherwise intact areas contained fine droplets of fat. Quite rarely a few mitotic figures in the myocardial fibers indicated beginning regeneration. The small blood vessels were often surrounded by inflammatory cells, and the endothelium was swollen. A few cellular foci were present in the connective tissue underneath the intact endocardial lining. The valves were not remarkable.

(*b*) Lungs: The alveolar septums generally were widened, edematous and infiltrated with mononuclear cells, occasional plasma cells and rare eosinophils. There were occasional small foci of necrosis involving the stroma, the alveolar lining and the cellular exudate in the lumens. The epithelial lining cells were large,

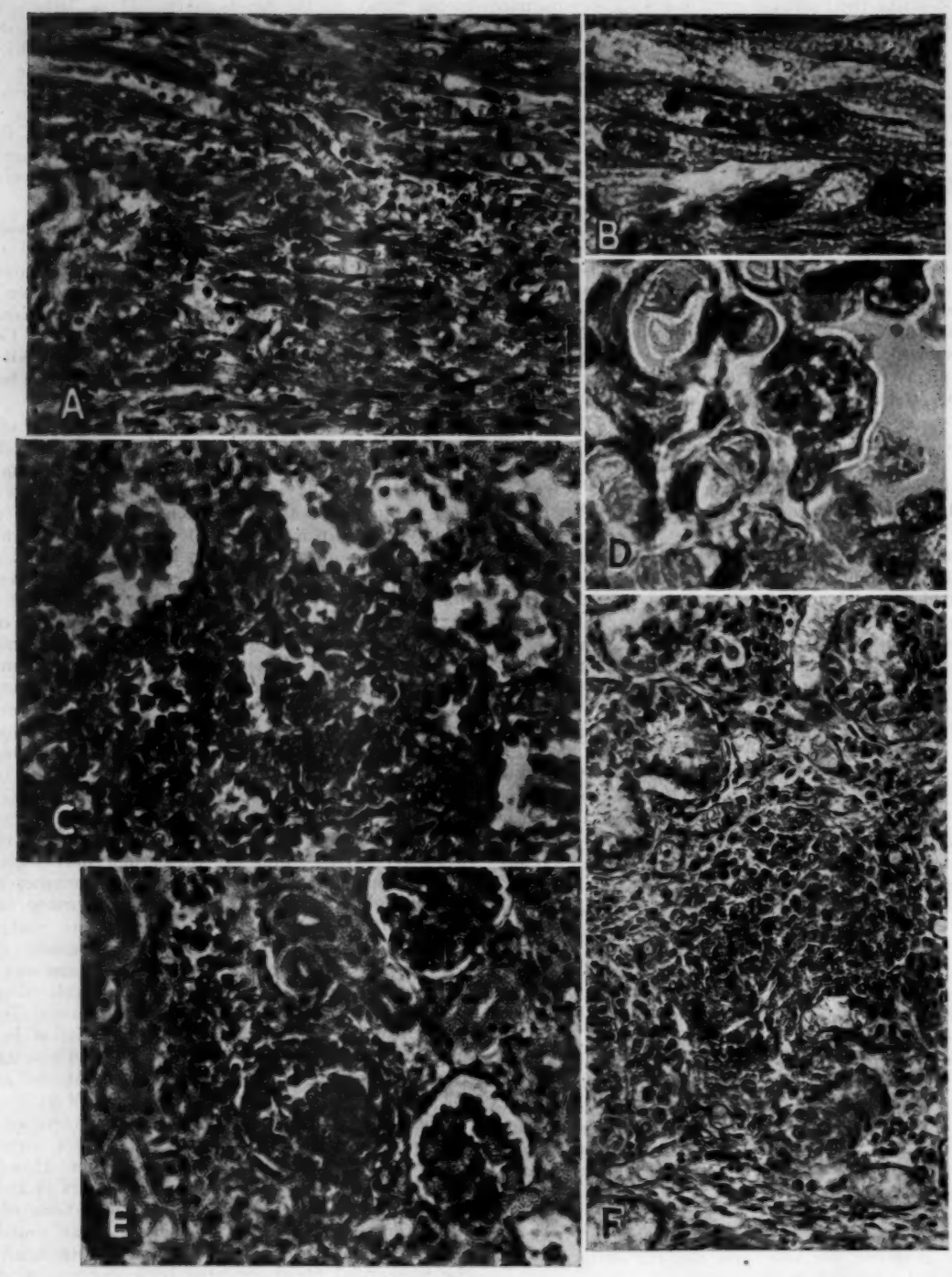



Fig. 1 (case 1).—*A*, myocardium showing an area of patchy necrosis and inflammatory cell infiltration. Hematoxylin-eosin; low power. *B*, myocardial fiber containing a group of toxoplasmas. Hematoxylin-eosin; oil immersion. *C*, infected lung. Note widening of the alveolar septums, hypertrophy of the alveolar lining, an area of necrosis in the alveolar wall and partly necrotic exudate in the lumen. Hematoxylin-eosin; medium power. *D*, parasites in an alveolar epithelial cell. Hematoxylin-eosin; oil immersion. *E*, focal glomerulonephritis. Note a partly necrotic glomerulus, exudate in the capsular space and infiltrations in adjacent tubules. Hematoxylin-eosin; medium power. *F*, testicle showing necrosis of a seminiferous tubule. The lumen is filled with debris and necrotic exudate. The adjacent tubules are intact. Hematoxylin-eosin; medium power.

swollen and hypertrophied (fig. 1 *C*), and many had become detached and were floating in the alveolar lumens, which in addition often contained large mononuclear cells with pale, finely foamy cytoplasm. Aggregates of parasites identical in appearance with those in the heart muscle were present in the cytoplasm of a few large mononuclear cells within the alveolar septums and in alveolar lining cells (fig. 1 *D*). A few single toxoplasmas were found free in the alveoli.

(*c*) Spleen: Fairly marked engorgement and a moderate degree of erythropoiesis were noted in the red pulp. The follicles showed karyorrhexis and occasionally necrosis and phagocytosis of nuclear fragments by large cells. Parasites were not demonstrable.

(*d*) Liver: The polygonal cells were often mildly vacuolated. The sinusoids were engorged. Neither necrosis nor inflammatory cell infiltrations were present, and parasites were not demonstrable.

(*e*) Pancreas: There were a few minute foci of necrosis of the acinous cells without a cellular inflammatory response. Parasites were not seen in these lesions.

(*f*) Kidneys: There were a few striking focal lesions in the cortex involving single glomeruli and the tubules and the interstitium in the immediate vicinity. In the fully developed lesions the glomerular tuft had undergone massive necrosis and constituted a round compact mass of debris and fibrin. In the capsular space there were collections of necrotic cells and fibrin. The epithelial cells of the parietal layer of Bowman's capsule were swollen or necrotic. The adjacent tubules were necrotic, and the area was infiltrated with mononuclear cells and plasma cells. In earlier stages of the glomerular lesion some of the capillary loops were still intact, while in others necrosis of the basement membrane and the epithelium was observed and the lumens were occluded by fibrin thrombi (fig. 1 *E*). In some of these partly preserved glomeruli, single parasites were found either in cells of the exudate within the capsular space or embedded in the necrotic remains of the capillary loop. The majority of the glomeruli were intact, but the capillary loops were often markedly engorged with erythrocytes. Here and there inflammatory cell infiltrations were noted about the arterioles of the cortex. There were also focal areas of necrosis in the medulla, involving the collecting tubules near the cortical border. In these lesions parasites could not be found.

(*g*) Adrenal Glands: In the well developed permanent cortex there were numerous small focal areas of necrosis involving the zona fasciculata. In such areas fibrin thrombi were sometimes noted in the lumens of necrotic capillaries. Usually no inflammatory cell response was present about these foci. Now and then a few single parasites were noted at the periphery of a lesion. In the loose, fairly wide boundary zone a few focal infiltrations with lymphocytes were present. The chromaffin tissue appeared intact.

(*h*) Testicle: Many of the seminiferous tubules had undergone necrosis (fig. 1 *F*) while adjacent units were well preserved. The lumens of the necrotic tubules were filled with debris and inflammatory cells, plasma cells, lymphocytes and mononuclear cells. The adjacent interstitium was usually infiltrated with similar cells for a short distance. In other areas the interstitium was the seat of extensive focal hemorrhages. Single parasites were occasionally found in the exudate within necrotic tubules. Intracellular collections of parasites were encountered in the spermatogonia of intact tubules (fig. 2 *A*). The parasitized cells sometimes showed beginning disintegration of the nucleus.

(*i*) Striated muscle: In sections of the psoas muscle parasitized fibers were found. The parasites were identical in appearance with those found in the myocardial fibers and were located beneath the sarcolemma sheaths in long tubular clear spaces with rounded ends (fig. 2 *B*). As many as 234 individual parasites were counted in one fiber. Such masses occasionally deformed the adjacent sarcolemma nuclei and usually displaced the sarcoplasm to one side. The affected fibers were swollen and had lost their striations, but as a rule no inflammatory reaction was noted. On the other hand, numerous small foci of edema, necrosis and inflammatory cell infiltration were present in areas where only a few single extracellular parasites could be demonstrated. The infiltrating cells were chiefly mononuclear, but there were plasma cells and a few eosinophils and lymphocytes. Now and then similar infiltrations were noted around blood vessels. In rare instances focal inflammatory lesions were found adjacent to heavily parasitized but unbroken muscle fibers. In fragments of small muscles present in sections of thyroid gland and ribs no parasites or lesions were found.

(*j*) Other tissues: Sections of the remaining somatic tissues failed to reveal significant lesions except for increased erythropoiesis in the bone marrow and depletion of lymphocytes in the lymph nodes. The umbilical vessels were free of inflammatory changes and parasites. The ganglion cells in visceral organs and in ganglions about the aorta and the adrenal glands, and the visceral nerves appeared normal.

(*k*) Brain: Twenty sections were examined, many of them serially. The areas selected for study included various portions of the cerebral cortex, the centrum semiovale, the corpus callosum, the basal ganglions, the hippocampus, the cerebellar cortex, the dentate nucleus, the pons and the medulla. Various stains were used, but hematoxylin-eosin was found most useful.

In the leptomeninges numerous inflammatory lesions were noted. These were mostly focal and were commonly located over adjacent lesions in the brain proper, especially in the depth of the cerebral sulci. They consisted of infiltrations with mononuclear cells and occasional plasma cells and eosinophils. The distribution of these cells was sometimes perivascular. Parasites were not found in the meninges.

The brain proper was the seat of innumerable lesions. No part of it seemed to have escaped, but the most severe changes were found in sections from the cerebral hemispheres, especially the cortical gray matter and the subcortical white matter. Two main types of lesions could be distinguished, although transitions between these types were often found. The lesion predominating in the hemispheres consisted of edema, loss of the fibrillar structure of the tissue, diffuse infiltration with compound granular cells and mononuclear cells, necrosis and often beginning cavitation (fig. 2 *C*). Where such lesions occurred near the surface, the cortex was slightly sunken, depressed below the level of the adjacent tissue. The glial elements in and about such areas were increased in number. Glial cells in mitosis were found fairly often. In the involved areas the nerve cells often showed signs of degeneration or necrosis, but in the remainder of the brain they usually appeared intact.

The blood vessels in the areas of destruction were almost invariably involved. The endothelial lining was swollen. Occasionally the vessel walls were necrotic, and here and there fibrin thrombi occluded the lumens. The Virchow-Robin spaces were infiltrated with mononuclear cells and sometimes a few plasma cells. In the perivascular areas edema and infiltration with compound granular cells were especially marked.

Parasites in large numbers, singly as well as in aggregates, were found in the destructive lesions (fig. 2 *D*). They were occasionally noted in the endothelial cells of blood vessels (fig. 2 *E*) or farther out in the vessel walls and once or twice in the lumens. They were abundant in the perivascular areas and in areas where softening and necrosis had begun but were rarely found in completely necrotic tissue. Crescentic forms were observed, but the majority of the organisms were ovoid. Besides single parasites, pairs showing axial symmetry and

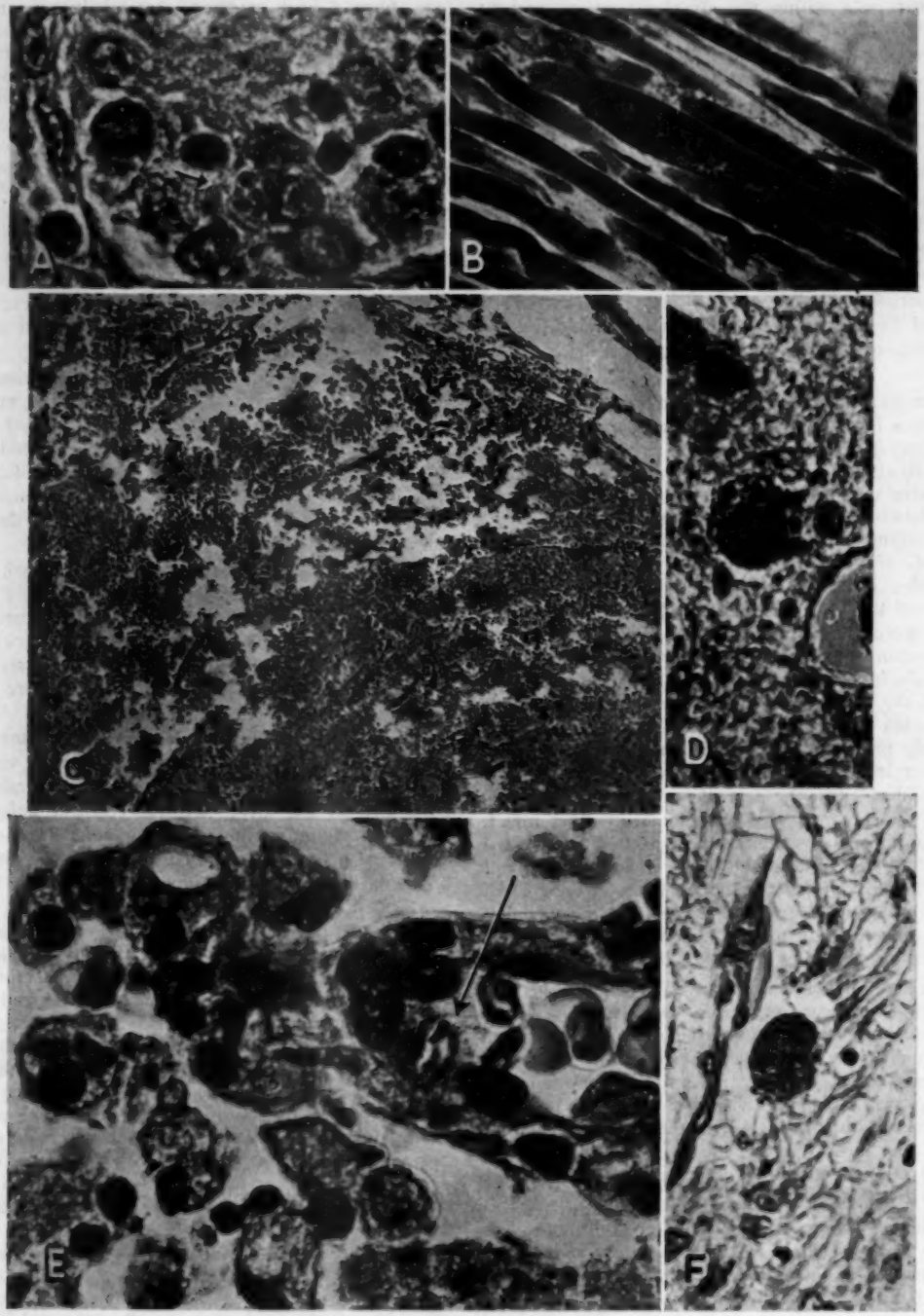



Fig. 2 (case 1).—*A*, testicle showing a group of toxoplasmas in an intact cell of a seminiferous tubule (arrow). Hematoxylin-eosin; oil immersion. *B*, striated muscle fiber containing a large aggregate of toxoplasmas. The adjacent fibers are intact. Hematoxylin-eosin; high power. *C*, area of destruction in the cerebral cortex showing loss of structure with beginning of a caving-in process of the superficial layers. Hematoxylin-eosin; low power. *D*, toxoplasmas aggregated (disintegrating host cell) and spreading extracellularly. Hematoxylin-eosin; oil immersion. *E*, toxoplasma in the endothelium of a cerebral vessel (arrow). Note also the perivascular infiltration with compound granular cells. Hematoxylin-eosin; oil immersion. *F*, "cyst"-like aggregate of toxoplasmas adjacent to a capillary in intact tissue (medulla oblongata). Hematoxylin-eosin; high power.

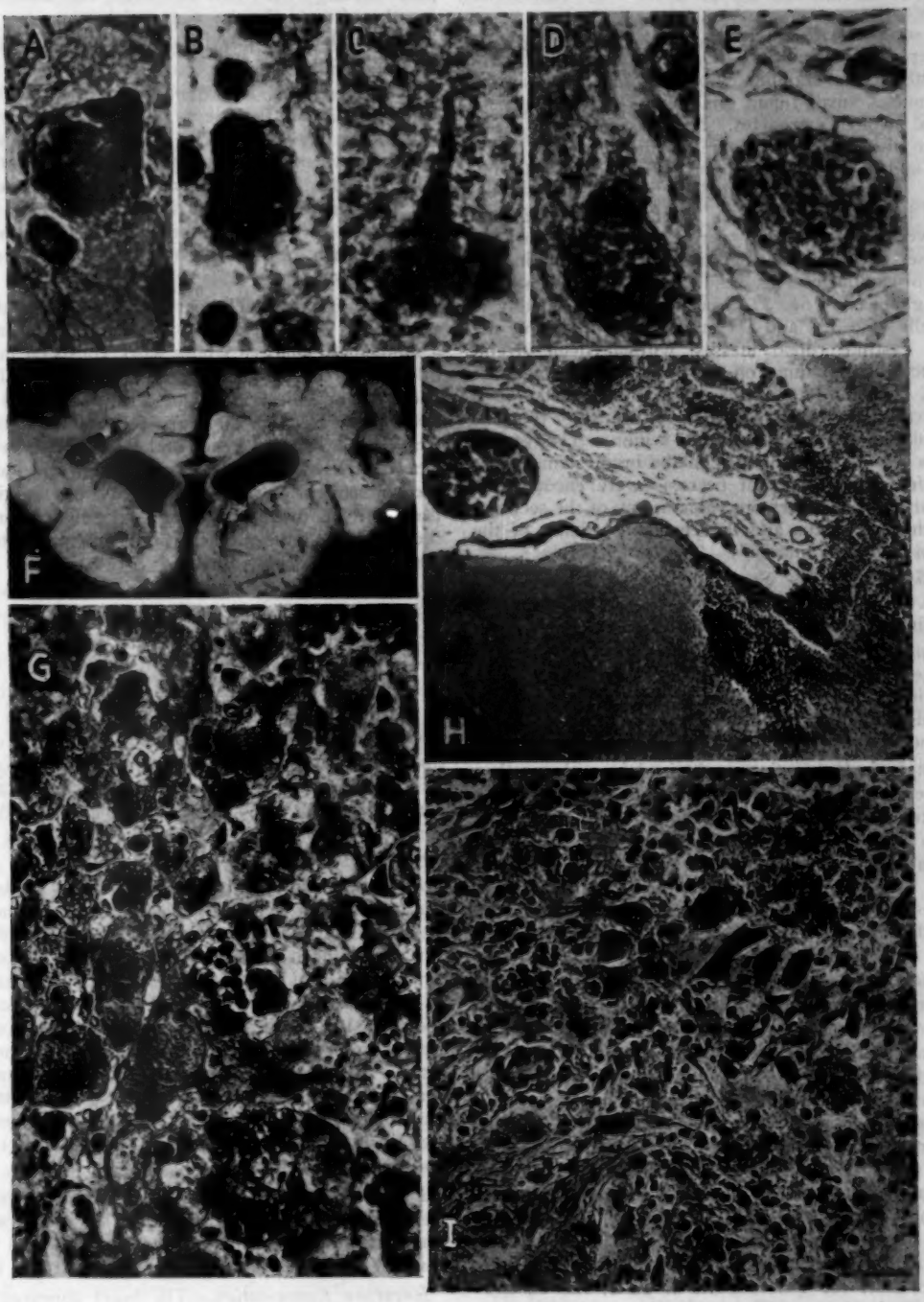



Fig. 3.—*A* to *E*, toxoplasmas in various cells in case 1: *A*, glial cell with nucleus displaced; *B*, glial cell; *C*, pyramidal cell of the cerebral cortex; *D*, Betz cell of a motor area; *E*, cell whose nuclear structure is lost. The cell outline is still distinct. Note absence of a tissue response. Hematoxylin-eosin; oil immersion. *F*, cut surface of the brain in case 2. Note multiple cavities, deposits in the lining of the lateral ventricles and dilatation of the ventricles. *G*, liver in case 2. Note swollen pale granular cytoplasm and fusion of many polygonal cells. A focus of normoblasts is seen in the center. Hematoxylin-eosin; medium power. *H*, cerebral cortex in case 2. A large area has been caved in. The space vacated by the breakdown of cortical tissue has been filled in by granulation tissue, calcified material and proliferation of the meninges. Compare this with the early stage of the process illustrated in figure 2*C*. Hematoxylin-eosin; low power. *I*, granulation tissue replacing cerebral cortex in case 2. Hematoxylin-eosin; high power.

small clusters of 3 and 4 organisms were often seen. Larger aggregates were seldom present in the destructive lesions.

The second type of lesion was granulomatous in character. It was miliary in size, composed of large cells with deeply staining cytoplasm and irregular, indented nuclei, small round glial cells, occasional plasma cells and swollen astrocytes. Necrosis was only rarely found in the centers of some of the larger of these lesions, and in these single parasites were often demonstrable. As a rule it was difficult to find single toxoplasmas in the granulomatous lesions, but large aggregates were often present peripherally or in nearby, otherwise normal-appearing tissue, especially in the vicinity of capillaries (fig. 2 *F*). These aggregates were seen in glial cells (fig. 3 *A* and *B*) and occasionally in nerve cells (fig. 3 *C* and *D*) or occurred in sharply delineated cystlike round masses, without demonstrable cell structure, containing on the average 50 to 60 toxoplasmas (fig. 3 *E*). The size of these "cysts" in a given area generally corresponded to the size of the cells predominating in that particular region. The granulomatous lesions predominated in the brain stem, where only a few small areas of necrosis were encountered. Calcification was not demonstrable in any of the sections.

The choroid plexus and the ependymal lining of the ventricles appeared intact.

(*l*) Spinal cord: Sections from various levels revealed the presence of numerous small granulomatous lesions similar to those predominating in the brain stem.

*Transmission to Animals.*—Maternal blood obtained under sterile precautions one month after delivery was injected intracerebrally and intraperitoneally into 8 adult white mice. The animals were observed for a period of five months but failed to show any evidence of illness.

*Immunologic Data.*—Serum obtained from the mother was frozen at — 40 F. two hours after removal and was immediately desiccated in the vacuum and kept in dry condition until used for neutralization tests on rabbits against a strain of Toxoplasma.[16] These tests were reported as showing no anti-Toxoplasma neutralizing antibodies in this serum. A second sample, obtained six months after delivery and treated in the same manner, also gave completely negative results.

*Summary.*—A white male infant described as apathetic since birth had repeated attacks of cyanosis, with convulsions and opisthotonos developing on the eighth day of life, and died on the eleventh day. At autopsy disseminated focal encephalomyelitis was noted, as well as a widespread myocarditis and focal myositis. Focal necrotic lesions occurred in the adrenal glands, the pancreas and the testicles. The kidneys were the seat of focal necrotizing glomerulonephritis. There was a peculiar type of interstitial pneumonia with hypertrophy of the alveolar lining cells. Parasites of the characteristic appearance of Toxoplasma were found in the brain and the spinal cord, the heart muscle, the skeletal muscle, the kidneys, the testicles, the adrenal glands and the lungs. The spleen was enlarged and was the seat of extramedullary hemopoiesis. An abnormality of the right eye was noted at autopsy, consisting in narrowing of the palpebral fissure, but the eyes were not examined ophthalmologically or pathologically. On two occasions, one month and six months after birth, the maternal serum was examined for neutralizing antibodies against Toxoplasma, none being demonstrated.

Case 2.—H., a Negro infant, one of identical twin boys, was admitted Sept. 5, 1943, because of prematurity. He was born at home by frank breech delivery five minutes after the birth of the other twin. The attending physician had difficulties in extracting the baby and had to slap his back in order to make him cry. Nevertheless the infant's color was satisfactory when he was brought to the hospital at the age of 30 minutes.

*Family History.*—The parents had lived in Mississippi until 1940, when they came to Detroit. The father was a municipal employee whose work consisted in cleaning alleys. In the performance of his duties he frequently had to handle dead rats and occasionally had to catch live ones. He was 26 years old and in good health. The mother was 25 years of age and likewise healthy. The family lived in a five room framework house in the Negro section of Detroit. The home at times had been infested by mice. There were no pet animals in the house, and the mother recalled no intimate contact with animals at any time.

*Prenatal History.*—The mother had borne 2 older children, now 4 and 2 years of age, both born at full term and apparently normal from birth on. She had never had a miscarriage. The last pregnancy had been uneventful until the fifth month, when she noted swelling of the ankles, lost her appetite and had attacks of dizziness and blurring of vision. She had not been aware that she was carrying twins. She volunteered the information that the fetus seemed considerably less active than her previous ones. The expected date of confinement was Oct. 17, 1943. The cause for the premature onset of labor was not known.

*Physical Examination.*—The infant was newborn, premature, fairly well developed and only moderately active. He weighed 3 pounds 11 ounces (1,618.5 Gm.). The crown-heel length was 16½ inches (42 cm.), the circumference of the head 11¼ inches (28.5 cm.), that of the chest 9⅜ inches (24 cm.) and that of the abdomen 10½ inches (26.5 cm.). The skin was covered with orange-colored vernix caseosa. The cranial sutures were widely separated, but the fontanel was not under undue tension. The heart, the lungs and the abdomen appeared normal. The infant cried vigorously when stimulated but otherwise acted sluggishly.

*Laboratory Data.*—The urine was normal except for the presence of rare white corpuscles in the sediment. The hemoglobin content of the blood was 21.8 Gm. per hundred cubic centimeters. The Kline test was negative.

*Roentgen Examination.*—The heart appeared slightly enlarged. The lungs seemed well aerated. Films of the long bones revealed multiple transverse bands of increased density near the diaphysial ends. The skull was not examined.

*Course.*—The patient was placed in the nursery for premature infants and given the usual care for a premature infant. A standard formula was given by means of a gavage tube. On the third day jaundice of the skin and the scleras was noted. There was at this time a transient fine punctate rash over the entire body. The patient was listless, and the temperature was widely swinging in character, ranging from 95 to

16. Dr. Joseph Heidelman, of the Wilmer Institute of Ophthalmology, Johns Hopkins Hospital, Baltimore, performed the tests for neutralizing antibodies against Toxoplasma reported in this article. His report on the serologic aspects of the cases reported here will be included in a separate article, which he is now preparing. The technic used by him essentially follows that of Sabin.[1]

102 F. Treatment with sulfadiazine was begun on the sixth day and maintained until death. On the eighth day the abdomen became distended, and the icterus increased. The child's respirations became somewhat labored, and he had attacks of cyanosis. During the second week two blood transfusions were given into scalp veins. The abdominal distention increased. The liver and the spleen were not palpable at any time. The stools were soft and yellow. During the third and fourth weeks the temperature rose to higher levels, the icterus increased and the stools were pale and at times clay colored. A yellow nasal discharge was observed. The child did not vomit but refused his formula. On October 5, one month after admission, he suddenly became intensely cyanotic, and respirations ceased. He revived temporarily after large quantities of bile-stained mucus had been removed from the nose and throat and was kept alive for another hour with injections of epinephrine hydrochloride and caffeine. In a similar attack shortly afterward he succumbed, at the age of 1 month.

*Gross Observations at Autopsy.*—A complete autopsy was made twelve hours after death. The body was that of a premature infant and measured 43.5 cm. in length. The skin was dry, inelastic, scaly, light brown. There was marked icterus of the scleras and the mucous membranes. The eyes were equal in size. The abdomen was markedly distended. The umbilicus was well healed. The anterior fontanel measured 2.0 by 2.0 cm. The cranial sutures were movable but not appreciably widened. There was icterus of the serous surfaces and vascular linings throughout the body.

The lungs showed a diminished degree of crepitancy and the visceral pleurae appeared edematous. On the cut surface the parenchyma had a mottled deep red and yellowish pink appearance.

The spleen was markedly enlarged and weighed 22 Gm. It was firm, deep brownish red on the cut surface, glossy and dry. The follicular markings were invisible.

The liver weighed 122 Gm. It was distinctly large, firm, with slightly rounded margins, and of dark brownish green color. On the cut surface the parenchyma was dark green, and the markings were indistinct.

The kidneys were not remarkable except for moderate icterus.

The adrenal glands were small. On the cut surface the cortex and the medulla were not clearly demarcated.

The long bones exhibited on the cut surface slight widening of the epiphysial lines without gross irregularities. In the midportion the cortex appeared somewhat thickened. The marrow was deep red throughout.

The subarachnoid space contained an excess of slightly turbid yellow fluid. The cerebral hemispheres were flabby and fluctuant and collapsed in the process of removal. The convolutions were well developed, pink in color. There were numerous bright yellow more or less sharply demarcated lesions on the cortical surfaces. These areas ranged from 0.1 to approximately 1.0 cm. in diameter and were slightly depressed; they were of approximately the same consistency as the adjacent brain tissue and produced a gritty sensation where entered with a wire loop. The brain was suspended in a 4 per cent formaldehyde solution after removal of pieces of cortical tissue for animal inoculation and for fixation in Zenker's fluid. It weighed 150 Gm. Coronal sections after fixation (fig. 3*F*) revealed numerous cavities, varying in size up to 1.4 cm. in diameter, occupying the white matter of the hemispheres, the cortex and the left caudate nucleus. The borders of these cavities were jagged and irregular, and the cavities were lined and filled with whitish or yellowish opaque material. None of the cavities communicated with the ventricular lumens. In the frontal lobes such white matter as was not involved in the process of cavitation presented a soft gelatinous, somewhat transparent appearance. In other areas the brain tissue appeared replaced by dry, slightly gritty material of a sulfur yellow or white color, without cavitation. Considerable amounts of such material formed irregular, interrupted bands underneath the apparently intact ependymal lining of the ventricles. The lateral ventricles were markedly dilated and surrounded in some areas only by a mere shell of cortex. The ventricular passages were patent. The choroid plexuses were somewhat firm, slightly gray and opaque, and in the lateral ventricles, were bound rather firmly to the ventricular surfaces. The patency of the outlets of the fourth ventricle could not be determined. The basal ganglions, save for some degree of compression, appeared grossly intact with the exception of the largely destroyed left caudate nucleus. No gross lesions were noted in the pons varolii, the medulla oblongata or the cerebellum.

The spinal cord showed on the cut surface numerous poorly defined whitish areas, and the gray matter was indistinct.

The right eye was removed intact for microscopic study and was fixed in toto in Zenker's fluid. Externally it did not appear unusual.

*Microscopic Observations.*—(*a*) Heart: There were occasional focal infiltrations of the interstitium with lymphocytes, mononuclear cells and rare eosinophils. The valves appeared normal. The myocardial fibers were uniform in size and well preserved.

(*b*) Lungs: The stroma was somewhat abundant but not definitely in excess of the amount expected in the lungs of a premature infant. In the alveolar septums mild edema and focal infiltrations with lymphocytes and unidentified cells with distorted pyknotic nuclei were observed. Here and there eosinophilic leukocytes and myelocytes were present. The lumens occasionally contained collections of macrophages.

(*c*) Spleen: There was a mild degree of hemosiderosis. The cellularity of the red pulp was increased because of the presence of moderate numbers of nucleated red blood cells and a few eosinophilic myelocytes. The follicles were preserved.

(*d*) Gastrointestinal Tract; Esophagus. The lamina propria was mildly infiltrated with lymphocytes. About the ganglion cells and nerve fiber bundles of the plexus of Auerbach focal collections of lymphocytes were present.

(*e*) Stomach: Here, too, mild lymphocytic infiltrations were found in the plexus of Auerbach. In the basal portion of the lamina propria of the fundus there were marked infiltrations with eosinophilic leukocytes.

(*f*) Pancreas: The stroma was slightly increased and edematous and contained numerous focal collections of hemopoietic cells. The acini and the islets of Langerhans were intact.

(*g*) Liver (fig. 3*G*): The lobular pattern was indistinct. The polygonal cells had undergone severe degenerative changes. The cytoplasm was swollen, pale, coarsely granular and often filled with irregular masses of bile pigment. Often there was fusion of small groups of cells into syncytial masses. Many of the nuclei had irregular wrinkled membranes, and in some cells the nuclei were no longer demonstrable. Definite necrosis was not observed, however. Within the cell cords

numerous small compact foci of normoblasts were found. The sinusoids appeared compressed and distorted. The Kupffer cells were swollen and contained granules of brownish or greenish pigment. The portal tracts contained a few hemopoietic elements. The bile ducts were patent and contained no secretion.

(*h*) Kidney: The epithelium of the convoluted tubules was somewhat pale and granular in appearance. In the stroma of the medulla and the pelvis there were numerous foci of hemopoiesis.

(*i*) Adrenal glands: The permanent cortex was well developed. The cells were depleted of lipid. No areas of necrosis or inflammatory changes were noted.

(*j*) Testicle: The epithelium of the seminiferous tubules was well preserved. There was marked focal hemopoiesis in the interstitium.

(*k*) Striated muscle: No lesions were seen in fragments of small muscles present in sections of the eye, the thyroid gland and a rib. In sections of pectoral, abdominal and psoas muscle occasional fibers were noted in which the striations had disappeared for a short distance and the nuclei of the sarcolemma had proliferated and formed beadlike rows. About such fibers and occasionally around small blood vessels mild infiltrations with lymphocytes and mononuclear cells were sometimes observed.

(*l*) Brain: Twenty sections stained with hematoxylin-eosin, Giemsa's stain, iron-hematoxylin, Masson's tetrachrome stain and von Kossa's stain for calcium were examined. In the areas corresponding to the gross lesions of the surface the cortical structure was completely destroyed and replaced by granulation tissue, necrotic or calcified masses and inflammatory cells (fig. 3*H*). Only a few typical, well preserved, single parasites were encountered in these lesions. The granulations were continuous with dense plaques of leptomeningeal infiltration and organization, and in such areas the surface was depressed below the level of the adjacent brain tissue. The granulation tissue consisted of capillaries, proliferating endothelial cells, histiocytes, multinucleated giant cells of bizarre shape, lymphocytes, plasma cells and rare eosinophils (fig. 3*I*). There was often extensive calcification of large necrotic areas. In the granulation tissue and in the adjacent brain tissue large mononuclear cells were often seen to contain intracellular deposits of calcified material in large individual granules or confluent masses of mulberry shape. The size and the shape of these deposits suggested that they were calcium-incrusted parasites. The surrounding tissue for some distance was infiltrated with mononuclear cells, compound granular cells, lymphocytes and plasma cells.

In the markedly thinned portions of the cortex the structure was lost even where no granulomatous replacement was found, but in the frontal and the occipital cortex it was usually preserved. The larger areas of cavitation sometimes appeared empty but usually contained amorphous calcified and necrotic material. The walls were lined by bandlike confluent deposits of calcium surrounded by granulation tissue and inflammatory cells. In some areas the structure of the brain tissue was extremely spongy and thinned out and consisted only of glial cells and scanty glial fibers separated by edema fluid.

The lateral walls of the ventricles, as a rule, had lost their ependyma and were lined by irregular bands of calcified and necrotic material beyond which there were glial cell proliferation and infiltration with inflammatory cells. Often short discontinuous bands of ependymal cells could be seen buried beneath masses of proliferated glia. In the mesial portions of the lateral ventricles these glial masses formed a large part of the surface. In the third and fourth ventricles and the aqueduct of Sylvius most of the ependymal lining was intact, but here and there small beetlike glial proliferations protruded into the lumens. Throughout most of the white matter there was a fairly marked increase in glial elements and swollen astrocytes as well as small round cells with compact round nuclei. In areas near large lesions in the hemispheres, calcium deposits were often noted in the media of small arteries. The vessels were often surrounded by infiltrations of plasma cells, lymphocytes and mononuclear cells in the Virchow-Robin spaces.

In the region of the brain stem smaller granulomatous areas, similar to those described in case 1, were the predominant lesions, and in these areas most of the structural pattern was intact.

Parasites were difficult to find. They occurred mainly as cystlike aggregates outside the lesions in essentially normal-appearing brain tissue. Only one such aggregate was discovered in examining numerous serial sections of the cerebral cortex. A few more were found in the brain stem.

The cerebellar cortex was free of inflammatory changes. In several lobules there was loss of the structural pattern. Small masses of cells similar to those composing the granular layer were surrounded by irregular branching extensions of the molecular layer, and Purkinje cells were missing in these areas or were scattered irregularly through the tissue. Such lobules were much smaller in size than the average and appeared to represent developmental anomalies.

The meninges were infiltrated with inflammatory cells, many of which were large macrophages with discolored, light brownish bulky cytoplasm. Here and there organization from the cortical surface produced adhesions between the pia mater and the arachnoid. The choroid plexus in two sections was free of parasites, and no lesions were demonstrable.

Smears of fresh brain tissue from a gross cortical lesion and from spinal fluid collected at autopsy were stained with Giemsa's and Wright's stain. Many mononuclear cells were seen to contain deep blue, fairly homogeneous round or oval intracellular bodies, often fused, corresponding to the calcified cellular inclusions noted in sections. Parasites were not demonstrable in any of the numerous smears.

(*m*) Spinal Cord: Sections from five levels were examined. Parasites in "cyst" form were more readily found than in the brain. There were a few lesions of miliary granuloma type, and the vessels in the cord proper and in the anterior fissure were often surrounded by cellular cuffs. The structural pattern of the gray matter was essentially preserved.

(*n*) Eye: The right eye after fixation in Zenker's fluid was embedded in celloidin. Sections at various horizontal levels were stained. There was diffuse infiltration of the ciliary body and to a lesser extent of the iris with inflammatory cells, mainly mononuclear cells but also neutrophilic and eosinophilic leukocytes. The iris was edematous. There was loss of pigment on its posterior surface. Throughout the retina there was an increase in Müller's fibers. Otherwise the retina appeared intact except for a large destructive lesion in the macular area. Here the retinal structure was completely destroyed and the underlying choroid was infiltrated with plasma cells, eosinophils, lymphocytes and mononuclear cells and was markedly edematous. Some of

the mononuclear cells had taken up pigment. Cell fragments and small amounts of pigment projected into the adjacent vitreous. Parasites could not be found in this lesion, but occasional "cysts" were noted just inside the layer of rods and cones in the intact portion of the retina. Inside the optic nerve mild edema and separation of fibers were noted. Around the arteria centralis edema and mild infiltrations with inflammatory cells were present. The extraocular muscle fibers appeared intact but focal, sometimes perivascular infiltrations with inflammatory cells were observed in the retrobulbar adipose tissue.

*Animal Inoculations.*—A piece of fresh brain tissue, approximately 1 sq. cm. in area, was removed under sterile precautions, ground in a mortar with approximately five times its volume of an 85 per cent sodium chloride solution and kept in the refrigerator until the following day, when 7 white adult mice were inoculated with it intracerebrally and 2 intraperitoneally. Ten mice of the same strain were kept as controls. None of the animals died or showed signs of illness over a six month period of observation. Several were killed, and no toxoplasmas were found in the tissues. Subinoculation into other mice failed to produce symptoms.

*Immunologic Data.*—Blood was obtained from the mother the day after the patient's death, one month after delivery. The tests for neutralizing antibodies against a strain of Toxoplasma were reported as strongly positive. The patient's own serum, which was not immediately processed post mortem, gave doubtful results in neutralization tests.

*Summary.*—A newborn premature Negro boy, one of identical twins, was observed from birth until death at the age of 1 month. During this time he was listless and had signs of obstructive jaundice, abdominal distention, evidence of poor regulation of body heat and episodes of cyanosis. No signs or symptoms indicating lesions of the central nervous system were noted at any time. At autopsy advanced encephalomyelitis with extensive destruction of brain tissue, replacement by granulation tissue, cavity formation, calcification and marked hydrocephalus were observed. In the brain and the spinal cord there were small numbers of parasites having the appearance of Toxoplasma. A few parasites were present in sections of the eye which also showed diffuse acute iridocyclitis and a sharply localized destructive and inflammatory lesion of the retina and the choroid in the macular region. There was mild interstitial bronchopneumonia. The spleen was enlarged. The liver was the seat of severe degenerative changes. Extramedullary hemopoiesis was present in the liver, the spleen, the pancreas, the kidneys and the testicles. There was a minimal amount of focal myocarditis with evidence of a residuum of focal myositis. Parasites were not found outside the central nervous system. Inoculation with tissue from a lesion of the brain failed to produce disease in mice. In the maternal serum one month after delivery neutralizing antibodies against Toxoplasma were demonstrable.

Case 3.—L. H., the twin brother of the preceding patient, was likewise brought to the hospital immediately after birth. This child had been the first to be born, also by breech delivery, and was the larger twin, weighing 4 pounds 8 ounces (2,041 Gm.) at birth. The crown-heel length was 17½ inches (44.5 cm.); the circumference of the head, 11⅞ inches (30 cm.); that of the chest, 10⅝ inches (27 cm.), and that of the abdomen, 10¾ inches (27.5 cm.). There was almost no vernix caseosa when the child was first seen. He was described as "not too active" but responding well to external stimuli. Except for a mild degree of separation of the cranial sutures the initial physical findings were noncontributory.

*Laboratory Data.*—The urine, first obtained on the sixth day, was normal. The hemoglobin content of the blood on the third day was 17.6 Gm. per hundred cubic centimeters.

Roentgenograms of the chest and long bones taken at the ages of 5 and 21 days revealed no abnormalities.

*Course.*—Like his brother, the patient became jaundiced on the third day of life. At that time there was a purulent discharge from the umbilical cord, which persisted for two weeks. The child was usually listless but ate fairly well. At the end of the first week a nasal discharge was observed. There were episodes of labored breathing, occasionally accompanied by cyanosis. The temperature was unstable at first. After the first two weeks elevations up to 100 F. were noted. The abdomen became moderately distended. The icterus persisted and even became more severe. Nevertheless, the child gained weight and was discharged on Sept. 30, 1943, at the age of 25 days. He was returned to the hospital on October 6, the day following the death of the twin brother, with complaints of diarrhea, anorexia and a cold.

In view of the results of autopsy of the twin, the patient was readmitted, and a thorough study was made.

He was now 1 month old. He weighed 5 pounds 9 ounces (2,523 Gm.). The circumference of the head had increased from 11⅞ to 12⅛ inches (30 to 30.8 cm.), but the cranial sutures were no longer separated. The icterus was still present, and the abdomen was distended. There was generalized desquamation of the skin. The heart and the lungs appeared normal. The reflexes were within physiologic limits. The child appeared listless and inactive.

The laboratory findings at this time were as follows: The urine contained bile but was otherwise normal. The stools were acholic and contained no bilirubin, urobilinogen or urobilin. The icterus index of the serum was 46. The van den Bergh reaction was biphasic in character. The hemoglobin content of the blood was 15 Gm. per hundred cubic centimeters. The white blood cell count was 8,800 per cubic millimeter.

The spinal fluid was xanthochromic, and a test for globulin was strongly positive. Sugar was present in normal amounts. The cell count was 500 red cells and 20 white corpuscles (all "mononuclears") per cubic millimeter. Smears of the sediment failed to show parasites.

Roentgenograms of the skull revealed multiple small areas of calcification apparently located in the brain substance.

The child was under observation until the time this report was written, that is until he was 7 months old. Much of this period he spent in the hospital. In the intervals he was followed in the outpatient department. During the third month of life the liver and the spleen became palpable. The abdominal distention disappeared sometime between the fourth and the fifth month. The jaundice persisted until the age of 5 months.

Repeated examinations of the spinal fluid revealed xanthochromia and an increase in globulin (278 mg. per hundred cubic centimeters on October 12). The spinal fluid did not become normal until the child was

4½ months old. At no time was it possible to demonstrate parasites in smears of spinal fluid. An encephalogram was made on November 13, at the age of 2 months. The films showed moderate dilatation of the lateral cerebral ventricles and suggested atrophy of the cortex, especially of that in the left hemisphere. There was no change in the extent of the intracranial calcifications.

During the first few months there were repeated episodes of fever with symptoms of an infection of the upper respiratory tract. On one occasion bilateral abscesses developed on the legs. One of these abscesses required surgical drainage. A pure growth of Streptococcus haemolyticus was obtained on culture of the exudate. During this time the patient was treated with sulfadiazine. On a later occasion he received sulfamerazine (2-sulfanilamido-4-methyl-pyrimidine).

Except for these intercurrent illnesses the patient did well and gained weight satisfactorily. Mild anemia developed. The mental development appeared somewhat retarded, but evaluation of the observations on a premature infant is difficult. The child learned to hold up his head at 5 months. At 7 months he was able to maintain a sitting position if his back was supported. He slept a great deal, and his mother thought that he was not as "bright" as her other children. At the age of 7 months he did not reach for objects but followed them with his head. By this time searching nystagmus had become evident.

Repeated examinations of the eyes were made (Dr. H. L. Begle). At the age of 1 month there was evidence of uveokeratitis. There was mild diffuse opacity of the corneas. The fundi were not clearly visualized. At 6 weeks the fundi were seen and appeared diffusely gray. The optic disks were indistinct. Localized lesions could not be made out. When the patient was 8 weeks old, the pupils reacted to light, but it was impossible to state whether there was vision. The child did not fix or follow objects with his eyes. The corneas were still slightly hazy. Threadlike extensions were noted running from the frayed borders of the pupils into the upper part of the pupillary space. The fundi were well visualized at this time and again gave a gray reflex. The disks were now sharply outlined. A few circumscribed white lesions were noted in the right macular area. At 5 months examination of the eyegrounds (Dr. Parker Heath) revealed definite patchy areas of chorioretinitis in both macular regions. At 7 months searching nystagmus was present. There was definite microphthalmos. The corneas were now clear, but remnants of the pupillary membrane were still present. The lens and the vitreous body appeared normal. In the macular region of each eye there was a single large white elevated lesion; that on the right extended to the margin of the nerve head.

The last general examination, on April 19, 1944, revealed a well nourished, fairly active child of 7 months weighing 13 pounds 9 ounces (6,152 Gm.). The circumference of the head had increased to 15¾ inches (40 cm.). The anterior fontanel measured 1.5 by 1.5 cm. The body length was 25⅛ inches (63.8 cm.). The temperature was 100 F. There was a slight hacking cough, but the heart and the lungs appeared normal. There was no jaundice. The liver was barely palpable. The reflexes of the upper extremities were not clearly obtained. The right knee jerk was distinctly hyperactive. There was a questionable tonic neck reflex when the head was turned to the left. The hemoglobin content of the blood was 10.6 Gm. per hundred cubic centimeters; the red blood cell count was 3,360,000 per cubic millimeter; the white blood cell count was 15,100. Roentgenograms of the skull showed the calcifications only faintly.

*Immunologic Data.*—A sample of serum taken from the patient at the age of 1 month was processed and sent to Dr. Heidelman for demonstration of neutralizing antibodies against Toxoplasma. The tests were reported as frankly positive. Inoculation of sediment from the spinal fluid, obtained on several occasions, into two successive generations of white mice failed to produce death or disease in these animals.

*Summary.*—A newborn premature Negro infant, the twin brother of a patient in whom toxoplasmosis was proved present at autopsy, was under medical supervision from the time of birth until the age of 7 months, when this report was written. Like his twin brother, he had severe icterus, which lasted for five months and was associated with acholic stools, bile in the urine, abdominal distention and enlargement of the liver and the spleen. Clinical evidence of Toxoplasma infection was obtained only when a special search was made at the age of 1 month. The spinal fluid was xanthochromic and its protein content elevated for at least four and one half months, while the pleocytosis disappeared within one month. Intracranial calcifications were demonstrable at the age of 1 month, and at the age of 2½ months there was mild internal hydrocephalus. Ocular changes were observed at the age of 1 month, but definite chorioretinitis was not demonstrated until the patient was 4 months old. By the seventh month searching nystagmus had developed. There was bilateral microphthalmos as well as persistence of remnants of the pupillary membranes. The mental development was definitely retarded although not markedly so in comparison with the performance of many premature infants. The physical development was as good as that of most premature infants. Neutralizing antibodies against Toxoplasma were demonstrable in the patient's serum, but attempts to transmit the infection to mice were unsuccessful.

## COMMENT

*Identity of the Parasites.*—In case 1 the diagnosis of toxoplasmosis rests mainly on morphologic criteria, namely, the appearance of the micro-organisms in sections of fixed tissue and the character and the distribution of the lesions caused by them. The organisms in the sections were well stained by hematoxylin-eosin. With Goodpasture's stain the cytoplasm appeared yellow and the nucleus light brown. The parasites were readily decolorized after being stained in carbolfuchsin. Crescentic and lanceolate forms were observed. The free parasites were larger than those found in the cystlike masses and were somewhat variable in size and shape. Their cytoplasm as a rule was homogeneous except for occasional basophilic granules, although a light perinuclear halo was sometimes observed. These features, according to Perrin,[17] are characteristic of Toxoplasma and make it possible to differentiate toxoplasmosis from the very similar Encephalitozoon infection. Perrin

17. Perrin, T. L.: Arch. Path. **36**:568, 1943.

also stated that, in contrast to the appearance of Encephalitozoon, the toxoplasmas in the "cysts" usually lack distinct individual contours. In the present case this was true in many instances but often the individual parasites were quite sharply delineated even in the cysts, and this was also noted in the illustrations published by Wolf and his co-workers from cases proved to be instances of toxoplasmosis by animal transmission and serologic tests.[18] It appears, therefore, that the presence or the absence of sharp contours of parasites within the cysts is not a reliable differential criterion but depends on the degree to which the host cell is crowded with parasites, the original size of the cell and the thickness of the section examined.

In case 2 the staining reactions and the appearance of the parasites were identical with those in case 1. The pathologic changes in the nervous system and in the eye were characteristic. The diagnosis was further established by the clinical findings in the twin brother and the demonstration of neutralizing antibodies against Toxoplasma in the latter's serum and in that of the mother.

*Pathogenesis.*—Several authors, notably Levaditi and his co-workers,[19] Sabin and Olitsky,[2] and Wolf, Cowen and Paige,[20] have studied toxoplasmic infection experimentally in animals. The genesis of the experimental disease has been summarized by Sabin.[1] The parasites first multiply locally at the site of the inoculation, then invade the blood stream, penetrate the blood vessels and localize in various viscera. They have a broad, somewhat variable range of tissue affinities. The distribution of the lesions depends not so much on the portal of entry of the infection as on the susceptibility of the different tissues in a given host.

In this respect the wide dissemination of toxoplasmas and of lesions due to their presence in case 1 is of interest. Only in one of the published cases of infantile toxoplasmosis was there a comparable degree of extraneural involvement. When the first reports of cases of human toxoplasmosis appeared, the impression prevailed that in infants the infection manifests itself mainly as encephalomyelitis and that visceral lesions, in contrast to those later found in adults, are uncommon and insignificant. The age of the patient seemed to be one of the chief factors determining this distribution. However, in several subsequent cases of infantile infection parasites were found in various organs besides the central nervous system, and in case 5 of Paige, Cowen and Wolf [6] the extraneural lesions predominated. These authors have proposed to distinguish three groups of cases of infantile toxoplasmosis, namely, those of encephalomyelitis, those in which visceral lesions predominate and those of generalized toxoplasmic infection without definite lesions. The observations in case 1 of the present report indicate that such sharp division may not be advisable since the central nervous system and other organs may be involved to an equal extent. It is becoming evident that the genesis of the natural disease in infants, as in adults, is strictly comparable to that of experimental toxoplasmosis. The organisms of toxoplasmosis reach the brain, as all other organs, through the blood stream. The distribution of the parasites and the lesions varies from species to species and may vary from individual to individual within a given species and probably varies with the strain of toxoplasmas. The age of the individual patient seems to be only one of several modifying factors. It is therefore to be expected that different combinations as regards distribution and severity of the lesions will be encountered as more cases of human toxoplasmosis are studied.

Nevertheless it seems that toxoplasmosis in human infants has a definite preference for the central nervous system. In all of the 14 pathologically verified cases of infantile infection (counting cases 1 and 2 of this report) the brain was involved, usually to a severe degree. The relative importance of the cerebral and ocular lesions is, however, magnified by the fact that, owing to the basic properties of nerve tissue, residual lesions are apt to be more conspicuous in the central nervous system than elsewhere. It is clear that even in cases of congenital infection the condition may have already passed from the acute into the chronic stage by the time the patient is born. In case 2 (in which the conception age of the patient at death was 9 months) and in several of the cases published by others most of the lesions of the brain represented stages in a healing process characterized by removal of necrotic debris, calcification, organization by granulation tissue and proliferation of glial elements. The extraneural changes produced during the stage of invasion may heal with less residue, depending on the extent of the original involvement and the ability of the specific elements of the various organs to regenerate. The regenerative capacity of fetal and neonatal tissues may be considerable. In case 1, mitotic figures were present in the myocardial fibers,

18. Wolf, Cowen and Paige.[3] Paige, Cowen and Wolf.[6]

19. Levaditi, C.; Sanchis-Bayarri, V.; Lepine, P., and Schoen, R.: Ann. Inst. Pasteur **43**:1063, 1929.

20. Wolf, A.; Cowen, D., and Paige, B. H.: J. Exper. Med. **71**:187, 1940.

indicating beginning regeneration even in the acute stage. In case 2, in which the brain was predominantly involved and the process was in a relatively late, chronic stage, minimal but distinct residual lesions were noted in the myocardium, and active regeneration was still demonstrable in some of the skeletal muscle fibers. Thus, in other organs residual lesions may be so slight and insignificant as to be easily overlooked, but in the central nervous system the extent of involvement and the inability of nerve cells to regenerate lead to more marked permanent damage.

*Distribution of Lesions and Parasites.*—Next to the central nervous system the commonest site of the infection appears to be the myocardium, which was affected in 4 of the 8 cases previously published in which complete autopsies were done and in both of the 2 fatal cases reported here. The lesion in case 1 consisted of widespread myocarditis with numerous focal necroses of the muscle fibers. The skeletal muscles were involved in both case 1 and case 2 of this report and lesions or parasites were reported in 3 other cases, while they were absent or not looked for in the remainder.[21]

Adrenal involvement is next in frequency, with necrotic lesions or parasites noted in 4 of the reported cases and in our case 1, while in the case of Torres [5b] the adrenal glands were not searched specifically.

As to the lungs, toxoplasmas were found in these organs in case 5 of Paige, Cowen and Wolf,[6] in case 1 of this report and in the case of Hertig restudied by Pinkerton and Weinman.[8] In the first 2 of these cases the changes consisted in diffuse interstitial bronchopneumonia with hyperplastic changes in the alveolar epithelium and a few foci of necrosis. The picture was essentially the same as that described in adults by Pinkerton and Henderson.[14] In Hertig's case there were no significant lesions.

In case 5 of Paige, Cowen and Wolf, lesions or parasites were encountered in the heart, lungs, adrenal glands, ovaries, skeletal muscle, umbilicus, subcutaneous tissue and thyroid gland. In our case 1 parasites were present in most of these organs but were not looked for in the subcutaneous tissues. The lesions in the testicles, observed for the first time in this case, correspond to those observed previously in the ovaries. Parasites were abundant in some of the seminiferous tubules.

Involvement of the pancreas and the kidneys likewise has not been reported in previous cases of human toxoplasmosis, infantile or adult. The pancreatic lesions were minimal in size and number, and parasites were not demonstrable. The renal changes consisted of focal acute necrotizing glomerulitis caused by the presence of parasites and associated with necrosis of adjacent tubules and a marked inflammatory response. In addition there were foci of necrosis in the collecting tubules of the medulla and occasional infiltrations about the preglomerular arterioles. Although not widespread, the glomerular process is of interest since it suggests that abnormal urinary findings may be encountered in cases of toxoplasmosis. Unfortunately, the urine in this case was not available for examination.

No parasites or lesions presumably due to the local action of the parasites were encountered in the liver or the spleen in either case 1 or case 2, nor have they been found in any of the published cases of infantile infection. This is surprising in view of the regularity with which lesions of these organs have been reported in cases of animal toxoplasmosis and in the 3 cases of infection of an adult.

In case 2 the only extraneural lesions were the minimal foci in the myocardium and skeletal muscles and inflammatory changes in the retrobulbar adipose tissue. Outside the central nervous system and the retina, parasites were not found in spite of a prolonged search.

*Portal of Entry.*—The observations in case 1 do not suggest a portal of entry for the infection. The absence of parasites in the liver would seem to argue against entrance of the parasites by way of the umbilical vein, but from what has been said about the tissue affinities of Toxoplasma it is clear that no definite conclusions can be drawn from negative data of this sort.

*Character of the Lesions.*—The lesions caused by Toxoplasma in the acute stage of the infection as seen in case 1 were basically similar in all organs except perhaps the lungs. They appeared to be modified by the degree of parasitic invasion and to a slight degree by the character of the tissue and were rather typical. They consisted of acute vasculitis, foci of necrosis of tissue followed by inflammatory cell infiltrations, and productive, granulomatous changes. In the earliest necrotic lesions inflammatory cells were

21. In this laboratory widespread chronic myocarditis of obscure origin was recently encountered in 2 infants, in whom foci of myositis were also observed in the skeletal muscles. There were no lesions in the brain, and parasites were not demonstrable in either case, but the myocardial lesions were in an advanced stage. Obviously, the diagnosis of toxoplasmosis could not be made in these cases, but the changes bore considerable resemblance to the lesions in toxoplasmosis and the coincidence of myocarditis and myositis in this age group is not easily explained by other conditions. Therefore attention is called to the possibility that Toxoplasma might cause myocarditis and myositis without producing lesions in the central nervous system.

as yet absent. The infiltrating elements were chiefly mononuclear cells and plasma cells, but eosinophils were often present. The granulomatous lesions, noted chiefly in the central nervous system, seemed to develop in response to small numbers of parasites. In some of the larger of these lesions, where necrosis of the centers was observed, larger numbers of free parasites were usually found. In areas of diffuse destruction parasites were nearly always present in considerable numbers.

Interestingly, in case 2, large destructive lesions, which predominated in the hemispheres, were almost absent in the brain stem, the spinal cord and the eyes, while active granulomatous changes were more numerous and parasites were more readily found in the latter sites. In case 1 the lesions were also more extensive and more destructive in the hemispheres than in the brain stem and cord. The question whether this was the result of a difference in vascularity or in susceptibility of tissues in the different regions cannot be answered at present.

In several of the cerebral lesions and in the adrenal glands and the renal glomeruli at least part of the necrotic changes appeared to be secondary to lesions of the blood vessels, supporting Sabin's observations on the lesions in experimental animals.[1] In general the changes were similar to, if not identical with, those in experimentally infected animals as described by Wolf, Cowen and Paige.[20]

The diffuse character of the pulmonary changes seems to contrast with the focal nature of lesions elsewhere. The explanation lies in the position of the lungs in the circulation. Since all the parasites on their way from an original focus to the various organs must pass through the alveolar capillaries, the lungs are exposed to greater numbers of organisms than any other single organ, and even though the susceptibility of the pulmonary tissues of infants to Toxoplasma appears to be relatively low, they cannot always escape the diffuse irritating effect of the parasites. This explanation is in keeping with Sabin's [1] concept of the genesis of toxoplasmosis and accounts for the identity between the pulmonary lesions in 2 infants (case 1 of this report and case 5 of Paige, Cowen and Wolf) and those in adults. The assumption that the degree of pulmonary involvement indicates the respiratory tract as the portal of entry does not seem necessary.

The character of the chronic lesions as seen in case 2 has already been referred to. Granulomatous changes were still present but were overshadowed by the large residual lesions with necrosis, calcification and organization. A comparison of the cerebral changes with those in case 1 indicates that most of the large lesions and cavities represent end stages of a softening process initiated at least in part by vascular changes. Destruction of brain tissue rather than obstruction of the ventricular passages seemed to account for the development of the hydrocephalus since the ventricular passages had remained patent in spite of marked glial proliferation in the wall of the ventricles proper.

*Relation of Parasites to Host Cells; Nature of "Cysts."*—Sabin and Olitzky [2] expressed the belief that Toxoplasma is an obligate intracellular parasite. The study of the cases reported here furnishes no conclusive answer to the question whether in man Toxoplasma is incapable of multiplying outside living cells. In case 1, pairs of parasites suggesting binary fission and small aggregates of 3 or 4 toxoplasmas were often found extracellularly in the vicinity of blood vessels in the brain, but in the other tissues multiplication invariably seemed to occur within cells.

According to Sabin,[1] the cystlike aggregates of parasites represent remnants of host cells invaded by the parasites and damaged to such an extent that the cell structure no longer is evident. The observations reported here definitely seem to support this contention. In case 1, in which an early stage of invasion was represented, the cell structures harboring the aggregates of toxoplasmas in the extraneural tissues, such as muscle fibers and epithelial cells, were always clearly recognizable, and the shape of the aggregate varied with that of the host cell. It is a priori unlikely that the parasites would behave in a totally different manner in the nerve tissues. Actually, many of the aggregates in the brain were found within nucleated structures which were still clearly recognizable as nerve cells and glial cells, and it was possible to demonstrate various stages in the loss of the characteristic cell structure to the point where the appearance of a cyst was produced. Additional, though indirect, evidence for the interpretation of the cysts as damaged tissue cells is furnished by the observation that the size of the "cysts" in the brain tissue in cases 1 and 2 generally paralleled the size of the cell type predominating in a particular location. Steiner and Kaump [10] were unable to find remnants of cell nuclei in a large number of "cysts" examined in serial sections. This and the observation of single extracellular parasites led them to suggest that the cysts may be products of the parasites themselves and do not use cellular substance of the

host for their further development. However, the infection in the case reported by these authors was in a relatively advanced stage, as shown by the presence of calcifications in the brain. In this stage invasion of host cells by the parasites no longer seems to take place, and the cells originally invaded during the acute phase have long since lost their nuclei. In case 2, in which likewise a late stage is represented, it was also impossible to demonstrate nuclei of host cells in any of the cystlike bodies.

Steiner and Kaump studied the relation between "cysts" and granuloma formation in the brain of their patient and concluded that the intact "cysts" do not produce a reaction in the adjacent tissue. The observations reported here seem to bear out this conclusion. The necrotic and inflammatory changes seem to occur as the result of the presence of free, single parasites in the vessels and tissues. Apparently this can take place when the parasites leave the blood vessels on their way to cells as well as after rupture of parasitized host cells,[3] when the parasites are again set free in the tissues.

The difficulty of finding parasites within necrotic exudate or in old lesions has been noted by several observers and was encountered again in cases 1 and 2 of this report. It seems that the inflammatory reaction leads to disintegration of the parasites. Wolf, Cowen and Paige [5] suggested that some of the organisms may become incrusted with calcium salts. The appearance and the arrangement of many of the calcified intracellular and extracellular granules in smears and sections of brain tissue in case 2 have led me to the same conclusion.

Eventually an equilibrium seems to be established between parasites and host either because the virulence of the toxoplasmas becomes attenuated or because the immunity of the host tissues increases so that even in advanced stages apparently viable intracellular aggregates can be found in otherwise normal tissue. This is in keeping with the observation of Weinman and Berne [22] that in experimental toxoplasmosis of mice with the organisms attenuated by sulfapyridine "cysts" persist indefinitely even though they cause no further symptoms.

*Prenatal and Postnatal Inception of the Disease.*—It is well established by the studies of Wolf and his associates, and others, that infantile toxoplasmosis may begin as an intrauterine infection. A number of cases with onset of symptoms within the first few days of life are on record. In 1 instance there was evidence of an advanced stage of the disease in a stillborn infant.[6] The demonstration of neutralizing antibodies against Toxoplasma in the serum of the mothers of most of the patients furnished additional evidence in favor of transplacental transmission. The demonstration of toxoplasmosis in cases 2 and 3 of this report is the first recorded demonstration of the disease in identical twins and contributes further proof that toxoplasmosis in human infants may have its origin in prenatal life. The lesions in the twin dying at the age of 1 month were well advanced, and those in the living twin presumably were in the same stage although not necessarily as extensive. Active lesions in the dead twin were limited almost entirely to the eyes, the brain stem and the spinal cord and were minor in comparison with the residual changes. The relatively slight activity of the infection explains the absence of symptoms referable to encephalitis. The prematurity and the lack of acute clinical symptoms in both twins, the advanced pathologic changes in the twin who died, the character of the ocular changes [23] and the demonstration of hydrocephalus and calcifications in the brain in the living twin indicate that the infection occurred in a relatively early stage of gestation, probably before the seventh month.

Neutralizing antibodies against Toxoplasma were demonstrable in the serum of the living twin and in that of the mother. The failure to demonstrate such antibodies in the serum of the dead twin must be ascribed to delay in collection and inadequate storage of the blood obtained twelve hours after death.

In case 1 there is no proof that the disease began in utero. On the contrary there is evidence to suggest that the infection was acquired in the first few days of life. The pathologic changes correspond remarkably well to those observed in animals one to two weeks after experimental inoculation with toxoplasmas as described by Wolf, Cowen and Paige.[20] The patient was 11 days old at death, and the assumption that the infection may have occurred during this time is entirely in keeping with the apparent age of the lesions. The failure of the tests to demonstrate neutralizing antibodies against Toxoplasma in the mother's serum on two occasions, three weeks and six months after the patient's death, likewise argues in favor of an acquired extrauterine infection in the infant. The possibility that the infection was acquired intra partum would require the assumption that the mother harbored toxoplasmas in the

22. Weinman, D., and Berne, R.: J. A. M. A. **124**:6, 1944.

23. The ocular changes in cases 2 and 3 will be the subject of a separate article in collaboration with Dr. Parker Heath.

vagina. In view of the repeatedly negative serologic tests this is unlikely.

It is of interest in this connection that in the 8 cases reported in detail by Wolf and his coworkers in which neutralization tests for maternal antibodies were made, failure to demonstrate them occurred only in instances in which prenatal inception of the disease was not indicated by other evidence (cases 1, 3 and 4 of series II [9]). The infection in those cases could have been acquired, and the lack of maternal antibodies would be in keeping with this. In the 4 cases in this group (cases 3, 4, 5 of series I [6] and case 2 of series II) in which the age of the patient at the onset and other clinical evidence indicated intrauterine inception of the disease, the tests for antibodies in the maternal serum always gave positive results. These facts lend significance to the absence of demonstrable maternal antibodies in case 1 of the present report and support the theory that the infection may have been acquired post partum.

Sabin has suggested that vectors may transmit the parasites of toxoplasmosis [1] and in 2 cases of infection of an adult [12] there was a history of tick bite. In my case 1 no history of bites was obtained, but the season (September) would be in keeping with the presence of vectors which might have transmitted the parasites.

*Clinical Features.*—The main clinical features of case 1 were convulsions, opisthotonos, instability of the regulation of body heat and disturbances of respiration. This picture corresponds to the description of early infantile toxoplasmosis given by Paige, Cowen and Wolf.[24] The changes in the spinal fluid (xanthochromia, mild pleocytosis and increased protein content) are like those reported in other cases. Hydrocephalus was not to be expected in the early acute stage. Hydrocephalus in toxoplasmic encephalitis follows obstruction due to granulomatous changes in the ependymal lining of the ventricular passages or extensive loss of brain tissue and indicates chronicity. The time element also explains the absence of calcification in the lesions of the brain.

The almost asymptomatic character of the infection in cases 2 and 3 has already been discussed. Had not the diagnosis in case 3 been suggested by the postmortem observations in the twin, the suspicion of toxoplasmosis would not have been aroused. Nystagmus at the age of 7 months was the first symptom. At that time some degree of mental retardation was evident, but the patient was a premature baby and his poor performance might be in part explained on that basis.

*Failure of Animal Inoculations.*—The failure to transmit the disease to mice by inoculation of brain tissue in case 2 and spinal fluid in case 3 may be explained in a number of ways. Sabin has pointed out the difficulties resulting from the low virulence of certain strains of Toxoplasma and has shown that several animal passages may be required before symptoms can be produced.[1] In the present cases, the material was carried through two passages, and none of the animals succumbed, nor were toxoplasmas found in the tissues when the animals were killed. In case 2 it was impossible to demonstrate toxoplasmas in smears of spinal fluid and of brain tissue from the same block, representing one of the gross lesions, which was used for animal inoculations. In case 3 smears of several samples of spinal fluid failed to show parasites. The conclusion appears justified that the failure of transmission experiments was due to the absence of toxoplasmas in the material used. This is in keeping with the absence of parasites in most of the advanced cortical lesions which were examined in case 2. Sabin obtained similarly negative results with biopsy material from the cerebral cortex, in which he was likewise unable to find toxoplasmas.[1]

*Icterus.*—A further point requiring discussion is the relationship of the icterus in cases 2 and 3 to the toxoplasmic infection. Icterus does not seem to be a common feature of the disease. Among 21 previously reported cases in which information on this point is available this symptom was present only in 3 instances. Interestingly, each of the 3 patients was newborn. There were 11 newborn infants among the 21 patients. Adding to these the 3 in the cases reported here but counting the 2 with icterus as 1 because the patients were identical twins, the incidence of icterus in newborn infants with toxoplasmosis is 4 in 13, or 30.8 per cent. The series of cases is as yet too small to establish a significant relationship between toxoplasmosis and jaundice on a statistical basis, especially since pathologic jaundice from a variety of causes is a common symptom during the neonatal period.

An analysis of the character of the icterus in the twins and of the underlying changes in the liver in the one who died does not lend support to the existence of a causal relation to the toxoplasmic infection. The icterus was of the regurgitation type and had its basis in a diffuse degenerative process in the liver. Focal inflam-

24. Paige, Cowen and Wolf.[6] Cowen, Wolf and Paige.[12]

matory lesions such as Toxoplasma has been shown to produce in the livers of adult patients and in those of experimental animals were absent, and parasites were not found. Thus a direct effect of the infecting agent on the liver appears unlikely. Sabin has expressed the belief that toxoplasmosis may produce general toxic effects,[1] and it is conceivable that icterus might occur on such a basis. Among experimental animals, however, jaundice was encountered only rarely in rabbits in the terminal phase of acute toxoplasmosis, and in this species the liver is directly involved by the infection. In my 2 cases the infection was in a chronic, almost latent stage, in which symptoms even on the part of the central nervous system were lacking, and the production of distant toxic effects would not be expected. Thus the possibility must be admitted that the hepatitis and the associated icterus in these 2 cases were coincidental and not related to the toxoplasmosis. Further observations are needed to clarify this point, which is of some importance since in case 2 the hepatitis rather than the toxoplasma infection appeared to be the cause of death.

*Extramedullary Hemopoiesis.*—In 4 of the 5 previously reported cases of toxoplasmosis with fatal outcome in the neonatal period in which adequate data are available, extramedullary hemopoiesis was found in the liver and the spleen and sometimes in other organs. Excessive numbers of nucleated red blood cells were found in the circulating blood in 2 of these cases. In 1 of them the observations in this respect were so impressive as to lead to the diagnosis of concomitant erythroblastosis fetalis.[10] Hemopoiesis was present in the enlarged spleen in case 1 and in the liver and the spleen in case 2 but the patient in this case died at a somewhat later period and had associated hepatitis. Discounting case 2, therefore, one finds the incidence of abnormal extramedullary hemopoiesis in this group is 5 in 6 cases, or 83.3 per cent. Enlargement of the liver or the spleen or of both, noted either during life or at autopsy, was found in 8 of the 11 adequately reported cases in this group. Again omitting case 2 and, for identical reasons, case 3, the observation of enlargement of the spleen in case 1 raises the incidence of enlargement of the liver or the spleen or of both in newborn infants with toxoplasmosis to 9 in 12, or 75 per cent. These data seem to indicate that extramedullary hemopoiesis and enlargement of the liver and of the spleen in these newborn infants with toxoplasmosis are related to the toxoplasmic infection. The formation of hemopoietic cells at sites other than the bone marrow is a commonly encountered, nonspecific response of the newborn organism to a variety of causes, among them infections. It is therefore not surprising to find this response in newborn infants with toxoplasmic infection, and a separate diagnosis of erythroblastosis fetalis based on such observations alone does not appear justified. Toxoplasmosis should be added to the list of conditions producing extramedullary hemopoiesis in newborn and young infants.

## SUMMARY AND CONCLUSIONS

Infantile toxoplasmosis is comparable to toxoplasmic infection produced experimentally in animals. Although in infants toxoplasmosis has a predilection for the central nervous system, the infection passes through a generalized stage in which many organs may be involved. The relative effect of toxoplasmosis on the central nervous system is magnified in later stages by the permanent character of the residual lesions, owing to the inability of nerve cells to regenerate, while lesions in other organs may heal with little or no residue. The disease may produce variable combinations of clinical and pathologic abnormalities.

The parasites of toxoplasmosis invade tissue cells, in which they multiply, causing gradual loss of the characteristic cell structure and producing the appearance of cysts. Various stages in loss of structure of the host cells from early invasion by parasites to the "cyst" stage may be seen.

The intracellular aggregate of toxoplasmas does not seem to produce a reaction in the tissue as long as the membrane of the host cell remains intact. Single parasites set free by rupture of a host cell or on their way from vessels to cells produce an inflammatory response. An equilibrium between host tissues and parasites may be established, leading to persistence of intact intracellular aggregates of toxoplasmas in normal tissues.

The first instance of toxoplasmosis occurring in identical twins is recorded. The demonstration of the disease in twins, together with the presence of neutralizing antibodies in the maternal serum and the chronic character of most of the lesions observed in the twin who died of the disease at the age of 1 month, constitutes new evidence for the occurrence of prenatal infections with Toxoplasma.

One patient, dying at the age of 11 days, had an acute generalized toxoplasmic infection. Lesions were found in many organs, among these the testicles, the pancreas and the kidneys, in which the infection has not been reported

until now. The renal lesion consisted of focal glomerulonephritis. Generally the changes resembled those in acute toxoplasmosis of adults previously described. The apparent age of the lesions and the absence of demonstrable antibodies in the maternal serum indicate the definite possibility that the infection in this infant was acquired after birth and suggest that even early infantile toxoplasmosis is not always necessarily congenital. The clinical onset of the disease in the first few weeks of life is not in itself adequate proof of its prenatal inception.

As to the significance of jaundice occurring in this disease during the newborn period, it is concluded that further evidence is needed before this symptom can be recognized as a manifestation of infantile toxoplasmosis.

The observations in at least one of the cases reported here and an analysis of cases reported by others indicate that hepatosplenomegaly and extramedullary hemopoiesis in the spleen, the liver and other organs are genuine manifestations of toxoplasmosis in newborn and young infants.

# PARATHYROPITUITARY SYNDROME

## (PITUITARY DYSFUNCTION AND "PRIMARY" HYPERPARATHYROIDISM)

ROBERT M. PERLMAN, M.D.

Assistant Surgeon (R), National Cancer Institute, National Institute of Health, United States Public Health Service

BETHESDA, MD.

Proofs for the occurrence of a parathyrotropic pituitary hormone have progressively accumulated in recent years. The following case of a nephritic male dog, 13 years of age, spontaneously presenting typical generalized osteitis fibrosa (von Recklinghausen) with coincident adenoma of the pituitary and parathyroid glands, embodies a particular, multiphased syndrome (parathyropituitary). Similar isolated cases of the syndrome in man have been reported in the medical literature. These were presented and their significance noted in an earlier paper.[1]

### REPORT OF A CASE

In the summer of 1941, a mongrel 13 year old male poodle dog was led into the clinic of Dr. Alfred Larue, a veterinary surgeon, in Geneva, Switzerland. The animal's master stated that it was no longer possible for him to care for the dog's increasingly frequent urination. Owing to a rubber-like consistency of the lower jaw, the dog had gradually lost the power to eat.

During the animal's short stay in the clinic, the veterinarian observed severe polyuria and urinary incontinence. The dog weighed 8 Kg. and was 25 cm. in height and 40 cm. in length. The feet were flattened out, and the animal had a tottering gait. There was what was described as "almost total blindness resulting from ocular cataracts." Marked polydipsia was manifest. The stools were irregular. Shortly before the animal's death, severe diarrhea developed. The abdomen was pear shaped and pendulous, and there was accompanying atrophy of the dorsal muscles, as well as abnormal, dark-colored pigmentation of the skin along the lateral abdominal surface. The dog was killed with strychnine.

*Autopsy* (only pertinent observations are herewith recorded).—A medium-sized dog was seen, with apparent normal skin and hair. A pedunculated fibroma, 3 by 2.2 by 1.5 cm., was located beside one of the right mammilae. The thoracic and abdominal adipose tissue was several millimeters thick and pale yellow.

Of the organs in the thorax and neck, the esophagus and the trachea apparently were normal; their mucosa was pale. The two lobes of the thyroid gland were light rosy tan and normal in size. A large adherent body, 10 by 9 by 3 mm., a giant parathyroid gland, was attached behind the lower left lobe of the thyroid gland and was deeper brown than the latter. An isolated cystic structure, 4 by 3.2 by 2.7 cm., friable in texture, was located to the left of the upper third of the trachea. On section it yielded a brownish serous exudate. The inner wall was slightly rugose, and the thickness of the capsule was not more than 1 mm. A small calcified solid tumor, the size of a kidney bean, was found in the wall of the cyst. Microscopic examination revealed that this body, as well as two others which were separated from the dorsal surface of the right lobe of the thyroid gland, were composed of parathyroid tissue (fig. 1 *A*).

The stroma of the thyroid gland was delicate, with no lymphocytosis. Some alveoli were filled with foamy colloid; others contained pale, stringy colloid, while still others were empty. In many regions there were centers of proliferation such as were described by Sanderson in certain cases of thyroid adenoma; however, there was not the histologic picture encountered in toxic diffuse goiter (Basedow's disease). Scattered solid masses of epithelial cells were seen, with some nuclear irregularity consisting of unusual chromatin structures. The follicular margins were calcified in many regions. Figure 1 *B* shows these thyroid nests in which one may see a certain proliferative activity. In one area of the thyroid gland a small parathyroid body was seen containing small empty vesicles in some areas and measuring 5 by 4.5 mm. on the section. A second parathyroid body, showing simple hyperplasia and measuring 7 by 4 mm. in a single nest measured in one section, was found in another region of the thyroid gland. In still another area a parathyroid adenoma, 8 by 3 mm., contained developing epithelial nests which were perivascular in location. These cells were small in size and tightly packed together. In this same adenoma, vesicle formations filled with bluish violet colloid could be seen in sections stained with hematoxylin-eosin. Finally, the peripheral stroma and the capsule were calcified. The thyroid tissue contiguous to this adenoma showed compression, and the follicles were flattened.

The tumor in the giant parathyroid body presented a lobular structure. Solid masses of cuboidal epithelial cells were observed. These nests of water-clear cells,

---

From the Institute of Pathology, University of Geneva, Switzerland.

The data in this paper were taken from the dissertation submitted by the author in partial fulfilment of the requirements for the degree of Doctor of Medicine (privately printed under the auspices of the Institut d'Anatomie Pathologique, Université de Genève, Ambilly, France, Imprimerie Franco-Suisse, May 1942). Some of the material from this thesis was subsequently published under the authorship of E. Rutishauser (Zentralbl. f. allg. Path. u. path. Anat. **80**:193, 1943), with the statement: "Die Arbeit erscheint ausführlich als Diss. Genf von R. M. Perlmann, 1942." (The work was published in detail as a dissertation by R. M. Perlman, Geneva, 1942.)

1. Perlman, R. M.: Syndrome hypophyseo-parathyroïdien, Geneva, Thesis, no. 1780, Ambilly, Imprimerie Franco-Suisse, 1942.

separated by a very vascular stroma, were formed principally by cells staining heavily for glycogen. Necrosis was not rare in the centers of these masses. Certain of the necrotic areas were almost totally calcified; other centers showed what were probably cholesterol crystals at the necrotic margin (fig 2 *A* and *B*). Fibrous capsules had developed around the centers of lipoid deposition and calcification. The entire tumor varied greatly in the amount of connective stroma. The first two parathyroid bodies (description already given) did not show this neoplastic structure with zones of epithelial degeneration and consecutive hemorrhage (fair-sized quantities of hemosiderin); instead, they presented simple hyperplasia. It should

The heart was globular in shape. There was extreme hypertrophy of both ventricles, with no dilation. The greatest diameter of the left ventricular wall was 2.2 cm.; that of the right ventricular wall, 5 mm.; that of the interventricular septum, 1.5 cm. The mitral valve ring was dilated, and the valve leaflets were thickened. The myocardium was firm. Microscopically, the myocardium showed marked hypertrophy and several necrotic foci with acute inflammatory cells. No metastatic calcification was seen in the slides examined.

Examination of the abdomen and the pelvis showed no ascites. The liver was large, extending 10 cm. below the costal margin. The spleen was visible in the center of the abdomen.

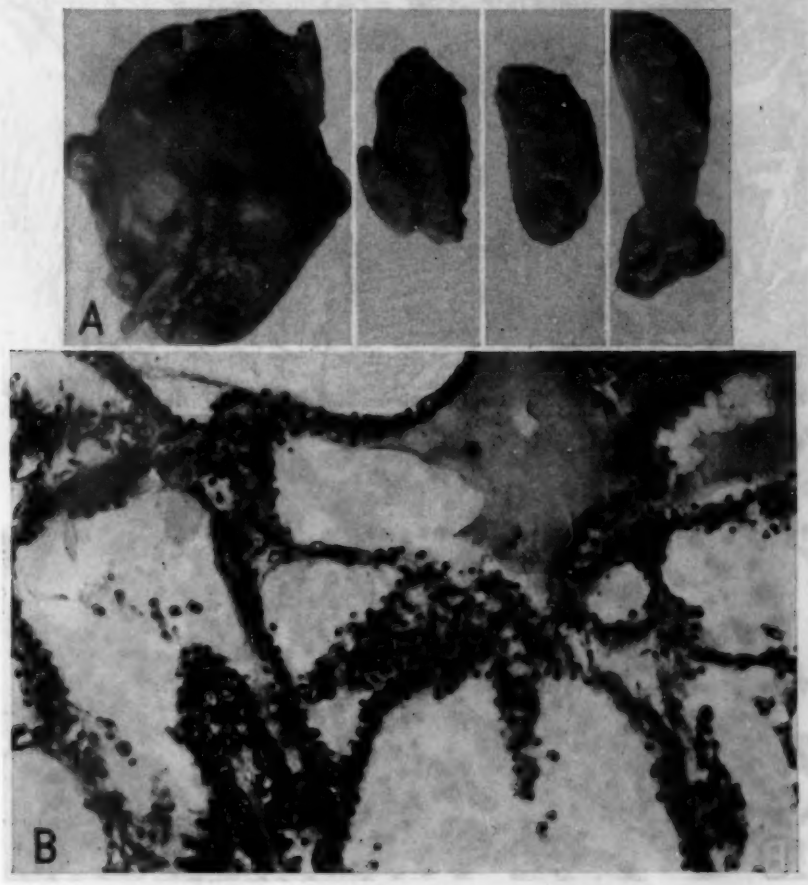



Fig. 1.—*A*, four parathyroid glands; the first two, at the left, are still attached to some thyroid tissue. *B*, thyroid gland showing centers of proliferation; hematoxylin-eosin stain; × 250.

be emphasized that, contrary to what takes place in adenomatous areas, parathyroid cells in regions of simple hyperplasia presented nothing but cords of "chief cells" in orderly arrangement. Interstitial lipomatosis, such as is often described, was nowhere found in these regions of parathyroid hyperplasia.

The lungs were apparently normal except for some edema and diffusely scattered spots and nodules which tended to be concentrated on or near the surface beneath the visceral pleura. Frozen sections indicated that these nodules contained calcium and were possibly an evidence of metastatic calcification. Microscopically, the lungs showed congestion, edema and some "heart failure cells." A few minute bone marrow emboli (attributed to violent cramps accompanying death by strychnine) were present in some of the pulmonary arteries.

The spleen was thin, measuring 12.7 by 5 by 0.7 cm. The capsule was wrinkled, and a small lobulated tumor, 0.8 by 0.6 by 0.4 cm., with a short, thick peduncle, protruded above the surface. On section the tumor showed reddish and grayish white zones which on microscopic examination proved to be angiomatous tissue with irregular confluent areas of necrosis. There was marked hemosiderosis of the splenic tissue. Megakaryocytes were frequent.

The liver measured 20 by 15.5 by 5 cm. and was friable. The surface of the right lobe was mottled with grayish anemic areas against a background of dark red. A similar but less pronounced condition appeared in other lobes. Microscopic examination disclosed that the grayish-appearing regions corresponded to hepatic cells stuffed with glycogen, while cells in

the dark red zones contained no glycogen. No icteric staining was noted. Microscopically, certain areas appeared hyperemic and others anemic. In the anemic areas, the hepatic cells were found rich in glycogen, while the protoplasm in other cells was poor in this substance. Cholangitis and pericholangitis were marked in regions of adenomatous hyperplasia. No definite cirrhosis could be diagnosed, although several large fibrous bands, almost free of evidence of inflammation, originated in Kiernan's spaces. In certain areas, glycogen was especially abundant in peripheral spaces of hepatic lobules. Almost no fat was seen in sections colored with sudan IV.

pepper grains corresponded microscopically to areas of hypertrophied pancreatic exocrine cells (exocrine microadenoma) containing more abundant protoplasm than was observed in other cells of the glandular portion of the pancreas. The nucleus was crowded to the base of the cell. The cytoplasm contained fine vacuoles and was lightly eosinophilic. The form and the distribution of the vacuoles suggested that these cells might have contained glycogen. The fact that these cells possessed distinctly marked boundaries would agree with this hypothesis. There was no evidence of inflammation. Islet tissue appeared slightly hyperplastic but was essentially normal.

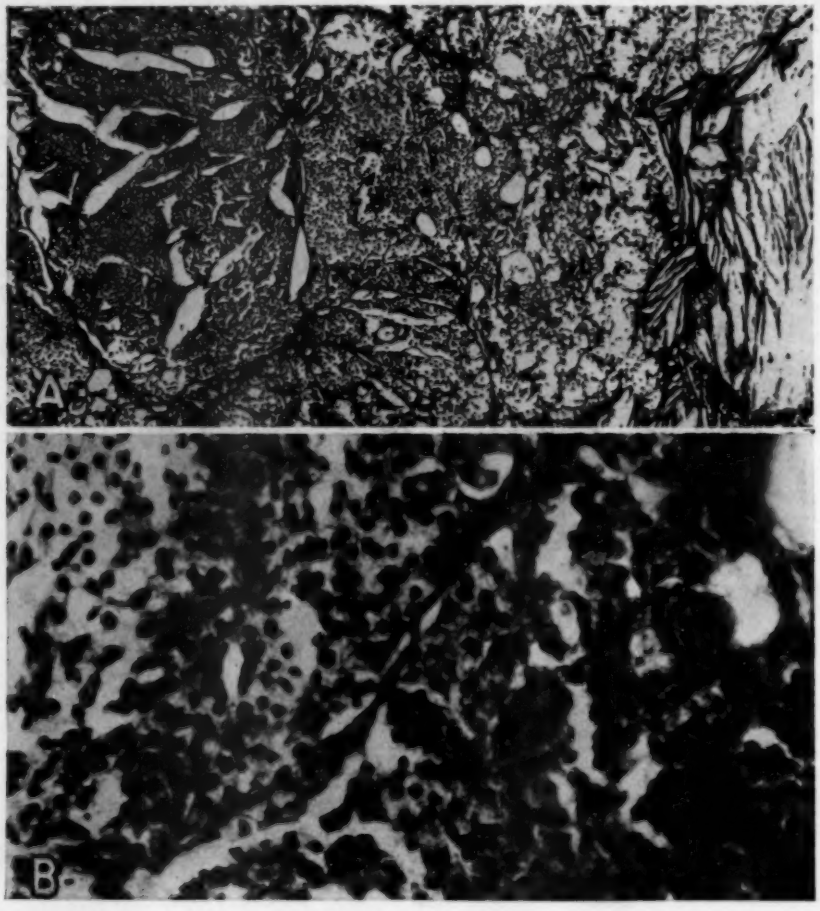



Fig. 2.—*A*, parathyroid adenoma with probable cholesterol crystal formation; Van Gieson stain; ×65. *B*, same adenoma; ×410.

The gallbladder measured 6 by 4 by 3 cm. and was thin walled; it contained no calculi. The bile ducts were patent; the bile was light green. There were no microscopic abnormalities.

The stomach was contracted, and the rosy mucosa was coated with mucus. No ulcers and no scars were seen. Microscopically, the mucosal parietal cells were clearly defined, possibly hyperplastic, often possessing two or even three nuclei. No calcium deposits were seen.

The duodenum and the small and large intestines showed no gross or microscopic pathologic changes.

The pancreas measured 14.5 by 3.2 by 1.0 cm. and weighed 31.5 Gm.; it was soft and rose colored. Several whitish opaque areas equal to the size of

The left kidney measured 5.5 by 3 by 2.5 cm.; the right, 5.5 by 2.5 by 2.5 cm. There was a scant adipose capsule. The thickened fibrous capsule detached with difficulty. The parenchymal surface was granular, with numerous gray-colored cysts of an average diameter equal to 2 to 3 mm. The cortex and the medulla were uniformly gray, pale and indurated, with no clear line of demarcation. The renal pelvis was not dilated; the mucosa was pale. The ureter was thin, not dilated. Microscopically, there were extensive interstitial sclerosis, atrophy of the papillae, some tubular dilatation and some swelling and necrosis of the tubular epithelium, with pronounced dilatation of the periglomerular spaces. There was slight thickening of the arterioles.

The bladder was normal.

The testicles showed no gross abnormality; they were not examined microscopically (lost at autopsy).

The prostate was not enlarged but contained several small adenomatous areas.

The left adrenal gland measured 4 by 1.7 by 0.6 cm.; the right was triangular, the two sides measuring 3 and 2 cm., respectively, with an average width of 1 cm. Both the glands contained many cortical tumors (adenoma) with a maximum width of 7 to 9 mm. These were extremely rich in lipoid. Microscopically, hypertrophy of the cortex was observed, with a very large zona glomerulosa and numerous cortical foci of adenoma. The medulla was large and slightly pigmented. No ganglionated cells were visible. Sympathetic ganglionic plexuses were seen frequently in fatty periadrenal tissue.

As to the pituitary gland, the sellar region was hidden by a small lobulated friable tumor which protruded over the anterior and posterior clinoid processes (fig. 3 *A*). The chiasma was flattened out. The tumor was elevated 0.5 cm. above the operculum. The latter was not differentiated with certainty from the surrounding tissue. The walls of the third ventricle were slightly caved in. The infundibulum, divided when the brain was lifted out, was filiform. The pituitary gland, removed from an enlarged sella turcica, measured 1.3 by 0.9 by 0.9 cm. It was composed of soft friable rosy

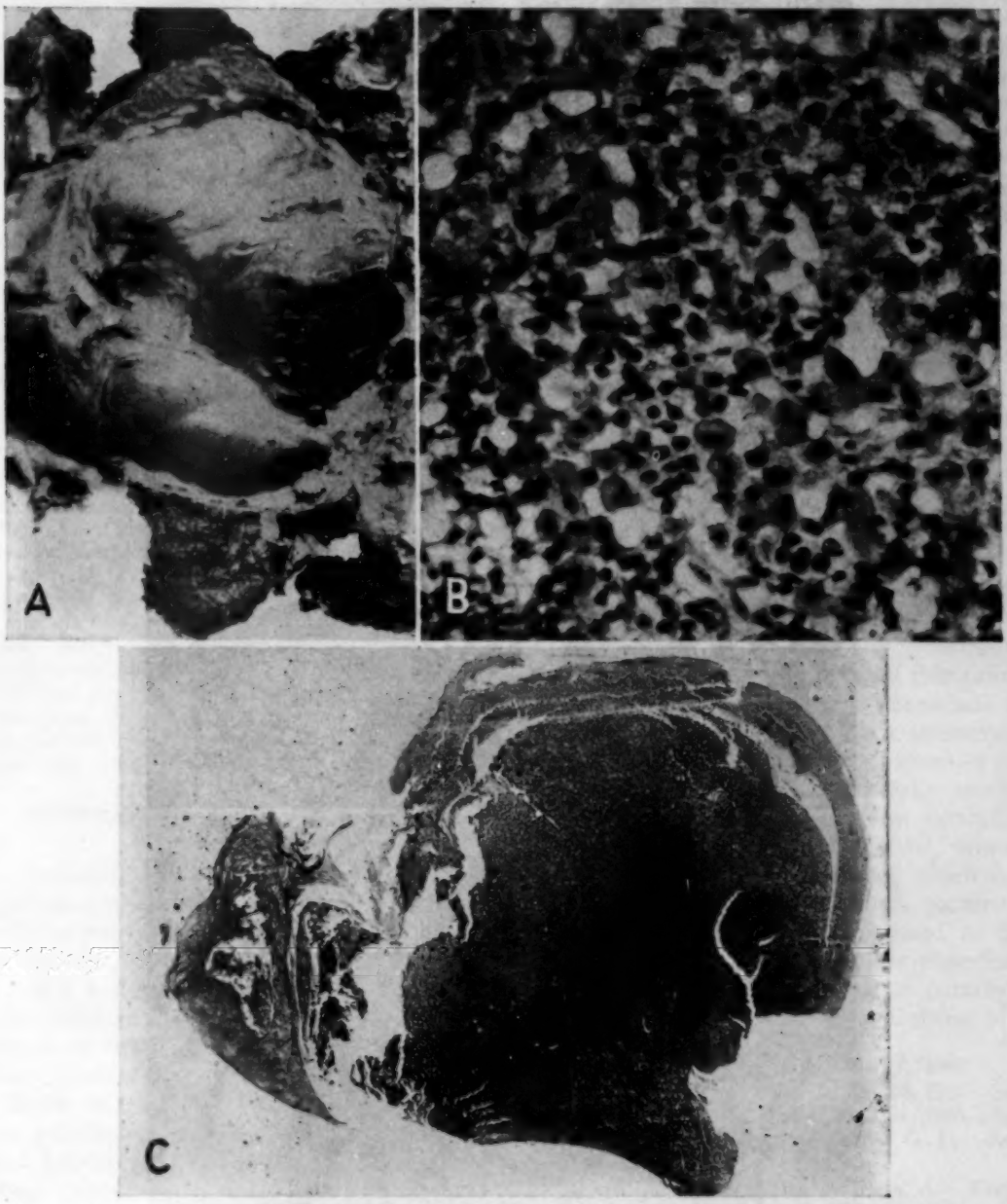



Fig. 3.—*A*, base of skull showing the optic chiasma and the pituitary tumor. *B*, atypical eosinophilic pituitary adenoma; hematoxylin-eosin stain; × 490. *C*, cross section through the longitudinal axis of the pituitary gland showing tumoral invasion of the anterior and posterior lobes; hematoxylin-eosin stain; × 9.

tissue, a bit redder in the center. The boundary between the anterior and the posterior lobe could not be determined macroscopically.

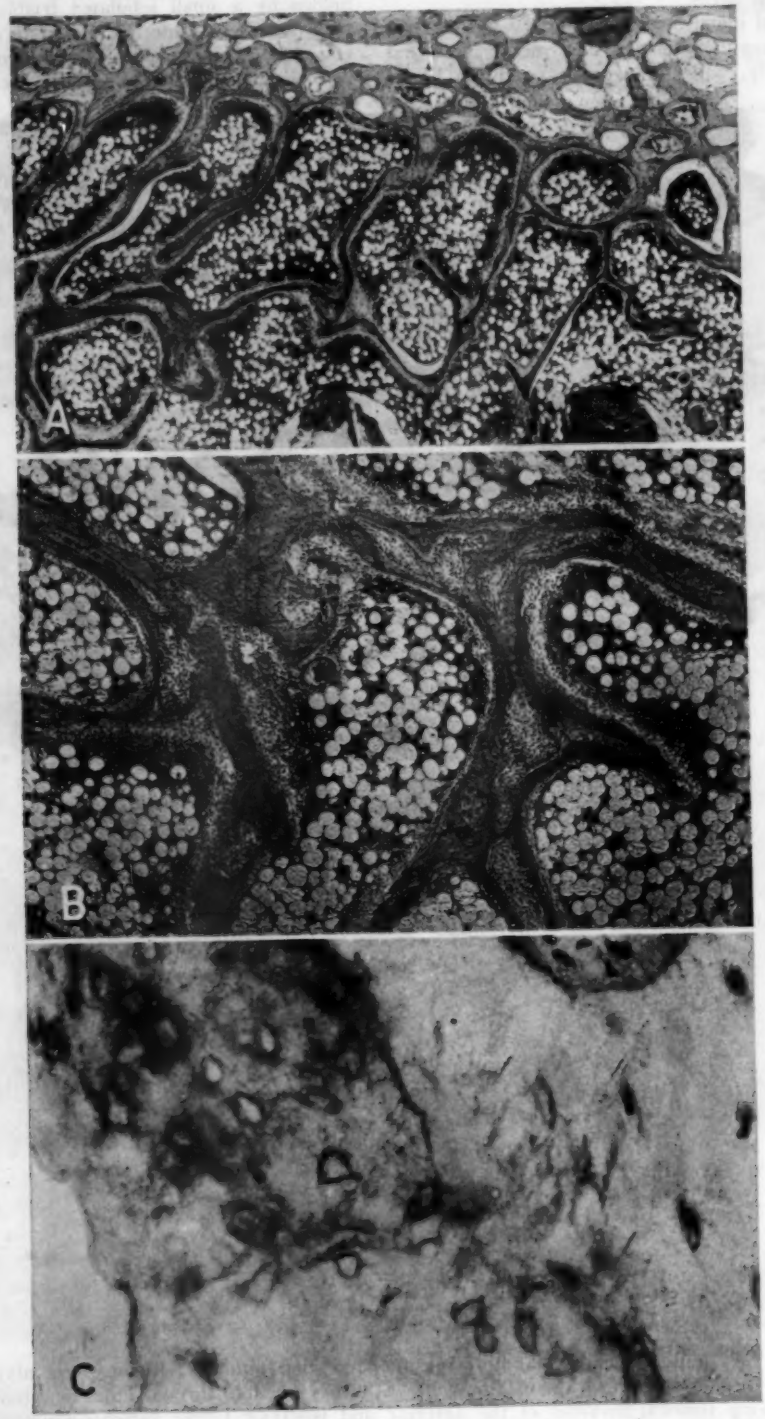



Fig. 4.—*A*, femoral epiphysis showing an active osteoclastic process and a pattern characteristic of von Recklinghausen's disease; Van Gieson stain; × 18. *B*, same; × 38. *C*, maxilla showing bone tumefaction where osteocytes are distributed irregularly and cells are uninhabited by nuclei; Van Gieson stain; × 490.

Microscopically, the anterior lobe of the pituitary gland was occupied by tumor tissue (fig. 3 *B*), retaining only in certain regions preexisting elements of the lobe. Eosinophilic cells were the essential constituents of the original structure; nevertheless, several basophilic cells persisted as well. The tumor was glandular in structure. The stroma of the tumor was vascular, with some areas showing deposition of hemosiderin and what were probably cholesterol crystals, probable evidence of

previous hemorrhage. The epithelial bands which formed the growth were composed largely of small cells resembling chromophobe cells, which under high power and oil immersion often showed a rosy protoplasmic halo. This tissue was strongly edematous; consequently, the cells were often connected only by protoplasmic bridges, which gave them a stellate appearance. The neurohypophysis was invaded extensively by these cells (fig. 3 *C*), which showed marked variation in size. The nuclei also varied, often appearing dense and deeply staining in some cells, pale in others. The chromatin was normal in some areas. The protoplasm, not abundant in the majority of cells, stained lightly with eosin in certain regions. Binucleated cells were observed from time to time. Their protoplasm appeared distinctly eosinophilic. Follicles filled with a pale eosinophilic substance were seen in a few regions. Sections stained according to the Tibor-Pap method showed the fine argentaffin fibers characteristic of endocrine tissue. The anatomic diagnosis was eosinophil adenoma, little differentiated. Only a few cells belonging to the original anterior lobe of the hypophysis could be recognized. The adenoma had infiltrated into the posterior lobe.

In the brain, aside from slight indentation of the third ventricle there was no gross abnormality. Slides of sections from the optic chiasma, the cerebellum and the frontal and parietal lobes were normal.

A résumé of observations on the skeleton includes the following: The mandible (temporomandibular region in particular) was very soft and pliable in every direction. The maxillas were also elastic. There was atrophic paradentosis. The long bones, unlike the jaws, were rigid and showed no diminution of resistance. Microscopically, grave osteitis fibrosa without brown cysts was present. Characteristic osteoid tissue was observed. Many bones showed a temporarily inactive state; elsewhere the osteoclastic process was active (fig. 4 *A* and *B*). Metabolic disturbances, influencing the osteocytes, led to regional disorders, including tumefaction and many bone cells which were devoid of nuclei (fig. 4 *C*).

## PROOFS OF PARATHYROPITUITARY RELATIONSHIPS

The probability of a pituitary influence over the parathyroid bodies received strong support from the animal experiments of Anselmino, Hoffmann and Herold [2] and other investigators who produced a systematic hyperplasia of the parathyroid bodies in dogs and rats by the administration of an extract of the anterior lobe of the pituitary gland. The hyperplasia proceeded to even double or triple the original size and weight of the parathyroid glands, with accompanying marked hyperemia. The assumption that these histologic changes were the expression of hyperparathyroidism was strengthened by experiments in which the blood calcium of the healthy animal was raised to a higher level after the introduction of the aforementioned extract, while this same effect failed to take place in the animal which had been subjected to parathyroidectomy. According to Schlegel,[3] Smith's results [4] lay in the same direction: He reversed the order of the foregoing experiments and noted that atrophy of the parathyroid glands followed hypophysectomy.

Jores and Seilnact [5] injected what was considered a purified extract of the anterior lobe of the pituitary gland into the cat, which increased the blood calcium, and wondered whether the osteoporosis in Cushing's syndrome as well as in acromegaly could not be due to the direct action of the anterior pituitary hormone on the skeleton, by regulation of the phosphate and calcium levels in the blood. Rivoire [6] cited five authors who demonstrated the stimulating action of pituitary extracts on the parathyroid glands. They obtained hyperplasia of these glands with an increase of blood calcium, which is probable evidence that substances stimulating the parathyroid glands are derived from the pituitary gland. Changes in parathyroid glands of rabbits described by Hertz and Kranes [7] resulted from injections of a pituitary substance and consisted in true hyperplasia with mitotic figures. Rivoire [6] stated that Cushing observed in the microscopic examination of the parathyroid glands of a patient of his with pituitary basophilism a histologic image absolutely comparable to that of the parathyroid glands of rabbits treated with the aforementioned extracts.

Hoff,[9] thinking of the possibility that primary hyperplasia of the parathyroid glands was able to provoke secondarily morphologic alterations in the hypophysis, injected enormous doses of parathyroid extract into rats and thereby produced considerable disturbances of calcium metabolism; serial sections of the pituitary gland, however, revealed no morphologic changes which could be attributed to the effects of the parathyroid extract. These results were opposed to those which Kikusawa [10] obtained in his experiments with rats, in which injections of parathyroid extract were accompanied by a decrease of the

2. Anselmino, K. J.; Hoffmann, F., and Herold, L.: Klin. Wchnschr. **12**:1944, 1933; **13**:45, 1934; Ztschr. f. d. ges. exper. Med. **97**:51, 1935.

3. Schlegel, B.: Med. Klin. **36**:617, 1940.

4. Smith, P. E.: J. A. M. A. **88**:158, 1927. Smith, P. E., and Foster, G. L.: ibid. **87**:2151, 1926. Smith, P. E.; Greenwood, C. F., and Foster, G. L.: Am. J. Path. **3**:669, 1927.

5. Jores and Seilnact, cited by Jores, A.: Klinische Endokrinologie, Berlin, Julius Springer, 1939.

6. Rivoire, R.: Les acquisitions nouvelles de l'endocrinologie, Paris, Masson & Cie, 1937.

7. Hertz, S., and Kranes, A.: Endocrinology **18**: 350, 1934.

8. Footnote deleted by the author.

9. Hoff, F.: Verhandl. d. deutsch. Gesellsch. f. inn. Med. **46**:441 and 459, 1934.

10. Kikusawa, T.: Zentralbl. f. inn. Med. **96**:589, 1938; Okayama-Igakkai-Zasshi **49**:2425, 1937; **50**:250, 1938.

acidophilic and an increase of the basophilic and chromophobe cells in the pituitary body. Extirpation of the parathyroid glands led to an increase of the acidophilic cells. The same results were obtained with injections of calcium which, in addition, were followed by a decrease in the numbers of basophilic and chromophobe cells.

## COMMENT

Albright, Sulkowitch and Bloomberg,[11] reviewing 6 cases of what was previously termed primary hyperplasia of the parathyroid glands, distinguished between compensatory and idiopathic hyperplasia of parathyroid tissue. Compensatory hyperplasia was referred to as the condition observed when there is an increased need for parathyroid hormone to maintain the serum calcium at a normal level. Idiopathic hyperplasia is the condition encountered in the authors' 6 cases, in which some unfathomed influence "is apparently driving the parathyroid tissue to produce more hormone than is required, with resulting hypercalcemia and all the sequelae of primary hyperparathyroidism." The nature of the influence was not determined by the authors, although they had previously indicated considerable circumstantial evidence which might suggest an overabundance of a pituitary parathyrotropic factor.

Boyd[12] wrote that slighter and less typical manifestations of hyperparathyroidism are much more common and more difficult to recognize than the classic picture of osteitis fibrosa cystica. The condition should be suspected in every case of renal calculus. Cushing[13] claimed that "even minute adenomatous tumors of the parathyroid glands and pancreatic islets may lead to serious constitutional derangements of hypersecretory type . . ." He suggested hyperactivation of the parathyroid glands in pituitary basophilism and partly attributed the osteoporosis and other changes to such hyperactivity. Atkinson's[14] discussion of the changes of the skeleton in cases of acromegaly reported in the literature up to 1936 revealed that Bertolotti considered that the sphenoid bone showed characteristic changes as revealed by roentgenograms in the dystrophies which are to be found in hypophysial dysfunction, both of acidophilic and of basophilic cells. In 584 cases of acromegaly the spinal column was normal in 16.4 per cent and altered in 83.6 per cent. Kyphosis was found in 70 per cent, kyphoscoliosis in 16 per cent, scoliosis in 8 per cent and so on. These, it appears, are important considerations which show similar influences at work in both pituitary acidophilism and pituitary basophilism.

A thorough inspection of Cushing's 12 cases of pituitary basophilism[13] disclosed an interesting variety of accompanying bone diseases described by the respective authors in terms ranging from "fibrous osteitis" to "gigantism of moderate degree." These descriptions recall the uncertainty which reigned until recent years in the differential diagnosis of the various osseous dystrophies. Hunter[15] pointed out how von Recklinghausen's work on osteitis fibrosa had been completely forgotten and that hyperparathyroidism was still being confused with osteomalacia in 1929. Cushing's paper[13] was written in 1932, when Collip's parathyroid extract and various endocrine substances from other glands and the disturbances of metabolism in diseases of the liver, the kidneys and other organs were just beginning to be appreciated as factors in the production of bone diseases. Ten of the 12 cases selected by Cushing were first described before 1925. A close reexamination of the only 4 cases (original descriptions of Mooser, Raab-Kraus, Anderson and Zondek) in which the parathyroid glands were mentioned raises a question regarding the true nature of the osseous disturbances, and the possibility of an origin in pituitary dysfunction transmitted via the parathyroid glands must be considered.

The idea that a primary metabolic alteration (pituitary) accompanied by secondary alterations of the parathyroid glands and bones is the principal etiologic factor underlying various pathologic manifestations of calcium and bone metabolism was expressed in an earlier paper.[1] Human cases presenting aspects similar to those of the nephritic dog described in this paper were cited to show that the parathyropituitary syndrome embodies a more or less intense polyglandular adenomatous condition, varying within wide limits depending on the stage of the severity of the respective disorders, and affecting the endocrine organs in both the acidophilic and the basophilic phase of the syndrome. When the syndrome is pronounced and distinct, the pituitary, parathyroid, thyroid, adrenal and pancreatic glands are almost invariably affected. The gonads, to the contrary, show signs of retrogression, involution, atrophy or dysfunction. It is often impossible to decide definitely where simple hyperplasia

11. Albright, F.; Sulkowitch, H. W., and Bloomberg, E.: Arch. Int. Med. **62**:199, 1938.

12. Boyd, W.: A Text-Book of Pathology, ed. 3, Philadelphia, Lea & Febiger, 1938.

13. Cushing, H.: Bull. Johns Hopkins Hosp. **50**:137, 1932.

14. Atkinson, F. R. B.: Endokrinologie **17**:308, 1936; **20**:245, 1938.

15. Hunter, D.: Lancet **1**:897, 947 and 999, 1930; Proc. Roy. Soc. Med. (Sect. Med.) **23**:27, 1929; (Clin. Sect.) **24**:40, 1931.

ends and adenoma commences in many of the aforementioned glands. The evidence tends to suggest that the degree or the extent of parathyroid hyperplasia, adenomatous formation or hypersecretion, together with all the natural consequences, would be directly linked to a sort of vicious cycle established between the pituitary and the parathyroid glands and accentuated in cases in which renal dysfunction also exists. It is reasonable to suppose that chronic nephropathy can accentuate "primary" hyperparathyroidism, for there is evidence of a relation between renal and parathyroid functions: Hyperparathyroidism can create nephropathy, with calcium metastases, while, inversely, nephropathy can occasion augmentation in parathyroid volume.

The condition of the nephritic dog presented in this paper is an example of the widespread effects which may result from a small lesion in an endocrine gland. The changes probably developed in this order: The eosinophilic adenoma of the pituitary gland caused adenomatous hyperplasia of the parathyroid glands and hyperparathyroidism, which in turn produced a disturbance in calcium and bone metabolism, and nephropathy. Owing to the disturbed renal function, the hyperparathyroidism was intensified, and the pathologic conditions were thus augmented.

## SUMMARY

A dog having atypical eosinophilic adenoma of the pituitary gland presented at autopsy coincident adenomatous hyperplasia of the parathyroid glands and grave chronic nephritis, together with fibrous osteopathy (von Recklinghausen) minus brown cysts. The case and experimental evidence support the concept of a parathyroid-pituitary relationship with consequent effect on the calcium metabolism.

NOTE.—While this paper was in press, an excellent review entitled "The Parathyroid Glands and Parathormone" was published.[16] The authors cited experimental evidence for and against the possible elaboration of a parathyrotropic factor by the pituitary gland.

16. Pope, A., and Aub, J. C.: New England J. Med. **230**:698, 1944.

# PROTEIN AS OXIDASE

MARTIN H. FISCHER, M.D., Sc.D., AND WERNER J. SUER, M.S.
CINCINNATI

We described in previous papers [1] how shelf preparations of fibrin or casein (themselves inert) are activated through treatment with a proper alkali so that they behave like equivalent concentrations of the "catalase" extractable from living cells. Thus was evidence added to the idea that the "ferments," "enzymes" or "catalysts" of biologic reactions are the proteins and that the living mass is not a system in which ferment "appears" but *is* ferment. Older support for these conclusions lies in the following: (1) The purest ferments ever isolated are crystallizing proteins,[2] while the least pure (sometimes claimed to be carbohydrates) cannot be freed of reaction for nitrogen; (2) all the heavy metal inhibitors of fermentation are protein "coagulants" (in more modern colloidochemical terms, protein desolvants); (3) the heat "sterilization points" (specifically 45 and 65 C.) of ferment mixtures in vitro or in vivo are identical with those at which the two classes of the proteins found in greatest mass in protoplasm, namely, the globulins and the albumins, suffer most obvious and most sudden "coagulation."

The succeeding paragraphs cite experiments which show how shelf preparations of protein (specifically the casein and the albumin of milk) may be made to function as oxidase.

## EXPERIMENTS

In demonstrating the presence of oxidase we employed the accepted standard of color change to violet or purple (generally interpreted as an oxidation to indophenol) in a mixture of alpha-naphthol and paraphenylendiamine hydrochloride. Since the two materials are of practically identical molecular weight, they were in all instances freshly mixed from freshly prepared equipercentage solutions. When first made (from a 1 per cent solution of the former in ethyl alcohol and a 1 per cent solution of the latter in water) a 0.04 per cent mixture is pale amber. It does not change in days when 5 cc. portions thereof are kept in ordinary test tubes without shaking. This indicates that oxidation from the air is trivial; for if this process is hastened by the presence of oxidase (such as that extractable from potato and many other vegetable and animal tissues) progressive darkening is apparent in an hour and is so great in twenty-four hours as practically to render the reaction mixture opaque.

Gelatinous sodium caseinate (prepared by neutralization of 12.5 Gm. of casein with 15 cc. of water and 10 cc. of normal solution of sodium hydroxide) *behaves qualitatively and quantitatively like so much oxidase.* When it is added to a series of tubes containing the dye reactants, deepening of color appears in a few minutes, the speed of the reaction increasing progressively with every increase in the amount of the caseinate added.

The following demonstration is typical. Enough of the dye reactants is placed in each of six test tubes so that a 0.04 per cent mixture is formed after dilution to the ultimate of 5 cc. To the first (as control) nothing is added; to the remaining tubes 1 to 5 cc. of a 5 per cent mixture of the caseinate just described in 0.9 per cent sodium chloride solution is added plus enough of the last-named solution to bring all the mixtures to equal volumes. (In other words, a hydrated proteinate is dispersed in a "physiologic" salt solution. The reasons herefor will be discussed.) A change toward blue begins almost immediately and increases with every increase in the amount of caseinate added, and with time. This is shown in figure 1. In enough time, however, these initial, easily discernible differences disappear, so that in twenty-four hours, for example, all the tubes present a practically identical depth of color. Thus is illustrated for this casein product that law of catalytic activity which states that while in shorter periods there is a relation between rate of change and concentration of the "ferment," the final quantity of change is independent thereof.

This activity of the caseinate depends on its state. Casein as such dropped into this dye mixture does not affect it. Activity appears when alkali is added. In biologic studies of oxidase the salt routinely employed is sodium carbonate. Added to shelf casein it acts similarly. The effect is commonly interpreted as a proper adjustment of the hydrogen ion concentration to the ferment reaction; actually something more profound happens: The sodium carbonate unites

From the Laboratory of Physiology, University of Cincinnati.

1. Fischer, M. H., and Suer, W. J.: Arch. Path. **27**:815, 1939; **27**:824, 1939.

2. Sumner, J. B.: J. Biol. Chem. **69**:435, 1926. Northrup, J. H.: J. Gen. Physiol. **13**:739, 1930.

with the casein, and it is the resultant hydrated sodium caseinate that acts as the catalyst.

All the nine test tubes in figure 2 had placed in them an identical quantity of casein and thereafter (except for the control tube on the extreme left) increasing fractions of normal sodium hydroxide. After the contents had stood together for an hour, a gelatinous mass formed in each tube. When, then, these mixtures were diluted with 0.9 per cent sodium chloride solution containing a standard amount of the dye reactants, the color changes observable in the photograph came forth. The amount of neutralization in the successive tubes equaled 20, 40, 60 and so on up to 200 per cent of the accepted neutralization value of casein. Note that at the end of three hours (when the photograph was made) the formation of indophenol had increased with every addition of alkali up to a maximum, after which it fell off. The transition tube contained enough alkali to equal one and two-tenths times the neutralization value of the casein.

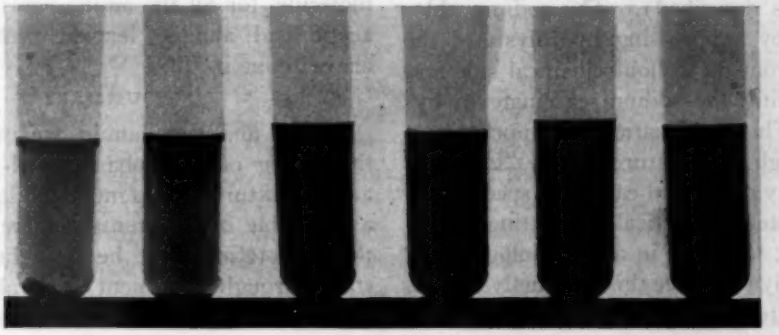


Fig. 1.—Increasing amounts of sodium caseinate have been dispersed in all the tubes except the first on the left. The progressive increase in depth of color change is to be noted.

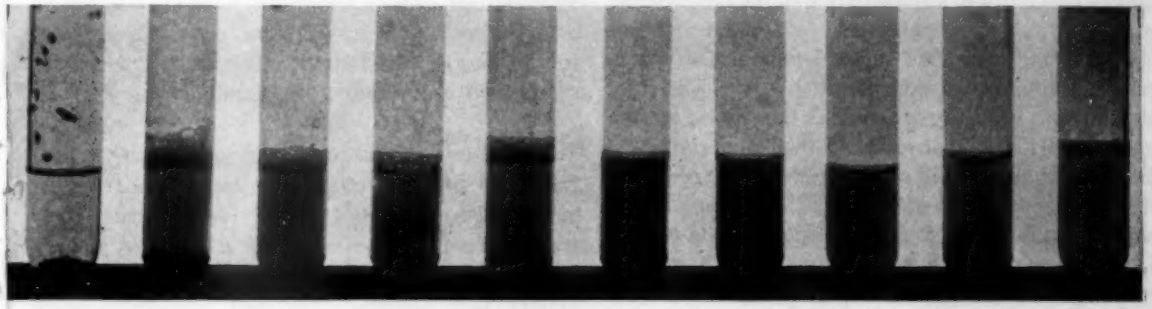


Fig. 2.—Equal quantities of casein have had increasing amounts of sodium hydroxide added to them except the first. Note the progressive increase in depth of color to an optimal region in the middle of the set.

Interpreted in terms of fermentation chemistry this experiment evidences that there is an optimum "alkalinity" for "oxidase" activity. The matter may be proved from the obverse side if one considers what happens when acid is added in increasing amount to an otherwise active sodium caseinate–dye mixture. Acid of every kind inhibits this oxidation exactly as when added to tissue oxidase. Enough acid suppresses all bluing. This proves true of lactic acid, too (which, as lactic acid is the principal acid formed post mortem, constitutes a fact that may be of service in explaining why animal tissues stop oxidizing themselves after death).

Sodium makes up the largest fraction of the basic elements combined with the proteins in the living cell. How does the substitution of ammonium, potassium, calcium or one of the yet heavier metals in the hydrated sodium caseinate employed as oxidase affect oxidation? The answer is that they all decrease it (in our opinion because the former yield proteinates more soluble in water; the latter, such as are less hydrated [3]). It does not matter how this substitution of one base for another in the proteinate is brought about. The basic proteinates may be made directly or they may be made indirectly by adding a proper neutral salt to a sodium caseinate. Inhibition of the rate of oxidation occurs in either case and in the order described.

And yet it is not mere increase in hydration capacity in the casein derivative over the pure

3. Fischer, M. H., and Suer, W. J.: Arch. Path. **20**:683, 1935.

substance that alone explains its elevation to a higher oxidative level; for if made from the acid side (casein hydrochloride, casein lactate and others) no bluing follows.

Extracts of oxidase from biologic sources are commonly declared to be "solutions" thereof in water. If sodium caseinate is regarded as a paralleling compound, then this conclusion seems unwarranted. The "ferment" needs instead to be thought of as the dispersion of a still hydrated colloid. Proof is found in the fact that dilution of an active sodium caseinate gel with water yields a less effective oxidizing system than dilution with 0.9 per cent sodium chloride (as in the experiments described). The sodium chloride acts (1) by suppressing hydrolysis of the caseinate and from the colloidochemical side and (2) by preserving the sodium caseinate in hydrated form. This explanation is supported by the effects of high temperature on any "ferment" or on the activity of sodium caseinate specifically. To boil it in water is practically to extinguish its oxidative value; to boil it in a "physiologic" salt solution is greatly to reduce this destructive effect.

In order not to lengthen these paragraphs, let it merely be stated that those "poisons" which affect biologically derived oxidases affect similarly this oxidative activity of sodium caseinate. All sulfides, for example, stop both. Of other materials studied under the conditions of experiment noted in foregoing paragraphs the following acted as inhibitors and in the order given: boric acid, oxalic acid, phenol and salicylic acid (even after due correction for the acid so added), formaldehyde, toluene. The last-named substance produced total inhibition.

Sodium lactalbuminate acts like sodium caseinate qualitatively; quantitatively, however, the rate of change to blue of an alphanaphthol-paraphenylendiamine mixture is only (about) half as great. The oxidative capacity described seems dependent on the integrity of the protein molecule, for all the amino acids tested (amino-acetic acid, alanine, leucine, tyrosine, histidine) are without it.

## SUMMARY

Casein and lactalbumin are made to catalyze the bluing of an alphanaphthol-paraphenylendiamine mixture like so much oxidase derived from a vegetable or an animal source. To this end, these protetins must be brought into a proper state through treatment with alkali; thereafter the effect of temperature, acid or alkali and the action of various poisons on the resultant system prove identical with what is observed when oxidase of organic origin is treated similarly.

# MORPHOLOGIC AND HISTOCHEMICAL STUDY OF THE EFFECT OF SCURVY ON TUBERCULOSIS IN GUINEA PIGS

## AND OF THE ORIGIN, AMOUNT AND DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE FOCI OF CASEOUS NECROSIS

WILLIAM O. RUSSELL, M.D.
AND
JAMES ALLAN READ, M.D.
ST. LOUIS
AND
LIEUTENANT ERNEST T. ROUSE
MEDICAL CORPS, ARMY OF THE UNITED STATES

More extensive disease has been shown in guinea pigs infected with tubercle bacilli and subjected to a deficiency of vitamin C than in control animals similarly infected and fed normal diets or diets containing large amounts of this vitamin.[1] The tuberculous lesions in the animals receiving vitamin C not only are less extensive but show a lessened tendency toward caseation, and there is a greater tendency toward the production of fibrous tissue around the individual lesions. The tendency of the animals receiving vitamin C to show more fibrosis around the lesions is in keeping with the known effect of vitamin C on the growth activity of fibroblasts. It can be supposed, therefore, that in the deficient animals it is the inability of the fibroblasts to produce normal collagen that is responsible for the lessened tendency toward fibrosis around the lesions.

It is interesting in regard to the general problem of the genesis of tuberculosis and the effect of a deficiency of vitamin C on this disease that guinea pigs, man and the higher apes all have poor natural resistance to tuberculous infection since these are the only animals that cannot synthesize ascorbic acid in their body organism and have scurvy if this substance has been removed for a time from the diet.[2] This suggests that vitamin C is concerned in a most fundamental way with the biologic mechanisms concerned in tissue resistance to tuberculous infection as seen in these three mammalian types. It is possible, therefore, that other mechanisms influenced by vitamin C besides the proliferation of fibroblasts and deposition of collagen may be concerned with the observed deleterious effect of a deficiency of this vitamin on the course of experimental tuberculosis. A factor influencing caseation would explain the more extensive caseous necrosis seen in scorbutic animals. Such a factor concerned with alkaline phosphatase appears not unlikely since alkaline phosphatase has been demonstrated in significant amounts in foci of caseous necrosis, and it has been shown that alkaline phosphatase in bone, kidney and serum can be influenced by a deficiency of ascorbic acid in a susceptible animal.[3] The studies of Gerstl and Tennant [4] demonstrating by experiments in vitro that guinea pig tissue lacks a phosphatase capable of splitting the tubercle phosphatid, such as that which is normally present in rabbit and mouse tissue, afford additional evidence suggesting phosphatase as a possible factor concerned with tissue resistance in tuberculosis. The histochemical methods of Gomori [5] and Takamatsu [6] for the demonstration of alkaline phosphatase in tissue sections have provided an unsual opportunity to investigate this problem. In this paper we report a morphologic and histochemical study of the effect of scurvy on experimental tuberculosis in guinea pigs and of the origin and distribution of alkaline phosphatase in the areas of caseous necrosis.

### EXPERIMENTAL PROCEDURE

Scurvy was produced in 10 young growing guinea pigs weighing approximately 300 Gm. by feeding a

From the Department of Pathology, Washington University School of Medicine.

1. Clausen, S. W.: Physiol. Rev. **14**:309, 1934. Grant, A. H.: Am. Rev. Tuberc. **21**:115, 1930. Greene, M. R.; Steiner, M., and Kramer, B.: ibid. **33**:585, 1936. Steinbach, M. M., and Klein, S. J.: ibid. **43**: 403, 1941.

2. Osborn, T. W. B., and Gear, J. H. S.: Nature, London **145**:974, 1940.

3. Thannhauser, S. J.; Reichel, M., and Grattan, J. F.: J. Biol. Chem. **121**:697, 1937. King, E. J., and Delory, G. E.: Biochem. J. **32**:1157, 1938. Harrer, C. J., and King, C. G.: J. Biol. Chem. **138**:111, 1941. Gould, B. S., and Shwachman, H.: Am. J. Physiol. **135**: 485, 1942.

4. Gerstl, B., and Tennant, R.: Am. Rev. Tuberc. **46**:600, 1942.

5. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.

6. Takamatsu, H.: Tr. Jap. Path. Soc. **29**:492, 1939.

ration known as rabbit chow checkers. This is a commercially prepared food for rabbits sold by the Purina Mills Company of St. Louis. The types of food materials used in its preparation, together with its complete chemical analysis, have been published elsewhere.[7] The laboratory department of the Purina Mills Company has been unable to identify any ascorbic acid in this ration by chemical analysis, and if guinea pigs are fed rabbit chow checkers exclusively, characteristic signs of scurvy develop and the animals die in from twenty-one to twenty-five days. One group of 10 control animals were fed lettuce ad libitum as a source of ascorbic acid. The inanition effect produced by the scurvy was controlled by a second control group of 10 animals, which received lettuce ad libitum but from which the rabbit chow was withheld and the animals selectively starved so that they lost approximately the same amount of weight as the animals not receiving lettuce. All of the guinea pigs received 0.5 mg. of virulent human tubercle bacilli of the H-160 strain of Corper, the inoculum being injected deep into the gluteal muscle of the right hindleg one day after they were placed on the experimental diets. Since guinea pigs will survive at best not more than twenty-five days when fed exclusively on rabbit chow, lettuce was fed to the animals deficient in vitamin C in two three day periods during the experiment in order to prolong their lives and allow adequate time for the development of tuberculous infection. All of the animals were killed forty-three days after being placed on the experimental diets, the vitamin C-deficient ones following a continuous eleven day period of deficiency. The guinea pigs of the latter group when killed were in extremely poor condition as a result of the deficiency of ascorbic acid plus the effect of the experimental tuberculosis. They had lost on an average 86 Gm. per animal; the animals in the inanition control group lost on an average 33 Gm., and the animals in the normal control group gained on an average 143 Gm. Two of the guinea pigs died several days before the experiment was terminated. These animals were not included in the study because of the possibility that postmortem autolysis significantly altered the alkaline phosphatase in the tissue.

## GROSS PATHOLOGIC CHANGES

*Deficient Animals.*—At necropsy the guinea pigs receiving the diets deficient in vitamin C were thin, and there was present but a small amount of subcutaneous fat. The right hindleg was enlarged and firm to palpation. Three of the 9 animals had a sinus tract in the right hindleg that drained a small amount of semifluid gray-white material from a cavity deep in the muscle. Section of the greatly enlarged right leg disclosed in the muscle tissue in all of the animals a large cavity filled with caseous material; in some animals it measured 2 cm. in diameter. A zone of mottled gray and red tissue, measuring approximately 2 mm. wide, formed the wall of the cavity and fused with the surrounding red-brown muscle. In the peritoneal cavity of every animal there was a small amount of light amber fluid. The lymph nodes in the right inguinal region were enlarged, and on section the normal lymphoid tissue was nearly completely replaced by large areas of gray-white caseation.

The liver and the spleen in each instance were markedly enlarged, and many irregularly outlined gray-white areas of caseation were noted on their surfaces varying in size from just perceptibly visible tubercles to much larger masses of homogeneous gray-white caseation, some measuring up to 2 mm. in diameter. The lungs had scattered, just visible, fine, gray-white tubercles on their pleural surfaces. On section small gray-white tubercles of the same type were seen in all parts of the parenchyma.

*Inanition Control Animals.*—The animals in this group were thin but did not show the advanced degree of inanition noted in the deficient group. None of the animals had a sinus tract in the right leg from the injection of the tubercle bacilli, although in all of them the leg was enlarged and firm to palpation. In the examination of the abdominal and thoracic viscera the only difference between the animals in this group and the deficient animals was that the tubercles seen in the spleen and the liver were generally smaller in comparison with those in the deficient animals.

*Normal Control Group.*—The animals in this group were not thin, and there was a moderate amount of subcutaneous fat present. One of the animals had a sinus tract in the right leg that drained a small amount of soft gray material. The gross pathologic changes in this group were essentially the same as those noted in the inanition control group of animals with the areas of caseation noted in the liver and the spleen, both being remarkably smaller than those noted in these organs in the deficient animals.

## HISTOLOGIC STUDIES

Duplicate blocks of liver, lung, kidney, spleen, adrenal gland and tissue from the lesion in the right gluteal muscle and the enlarged right inguinal lymph nodes were taken. One set of blocks was fixed in Zenker's fluid, and sections were prepared and stained with hematoxylin and eosin and with Masson's trichrome stain. The second set of blocks was fixed in 95 per cent alcohol, and paraffin sections were prepared for the histochemical demonstration of alkaline phosphatase as described by Gomori[5] and Takamatsu.[6] The demonstration of alkaline phosphatase by this method is based on the deposition of tribasic calcium phosphate ($Ca_3[PO_4]_2$) at the site of enzyme action in the tissue when a section of tissue is incubated with an organic phosphatate ester at a $p_H$ of 9 in the presence of calcium ions. The following procedure was used in order to insure optimum activity of the enzyme under properly controlled conditions. The paraffin was removed from the sections with xylene and the xylene with absolute alcohol. Following a dip in dilute collodion and drying, the sections were hardened in 90 per cent alcohol and washed with distilled water. Alcohol under these conditions does not affect the stability of the enzyme. The sections were then incubated in the substrate solution at 37 C. for two hours. The control sections were taken serially and placed in a dilute (0.1 per cent) calcium nitrate solution in tap water. Stock solutions were prepared, consisting of 3.2 per cent sodium glycerophosphate, 2 per cent calcium nitrate, 10 per cent sodium barbital and tenth-molar magnesium sulfate.

The final solution used had a $p_H$ of 9 and was made by diluting 6 cc. of sodium glycerol phosphate, 9 cc. of calcium nitrate ($Ca[NO_3]_2$), 6 cc. of sodium barbital and 6 cc. of magnesium sulfate ($MgSO_4$) to 60 cc. to give a final solution which was tenth-molar with respect to sodium glycerophosphate and magnesium sulfate. Following incubation, each section was placed in the solution with its control section, and both were stained for calcium by the von Kossa method. This stain depends on replacing calcium phosphate by metallic silver, thus giving a brownish coloration to the place where the calcium phosphate has been deposited. The site of activity of alkaline phosphatase is then marked by a brownish black

7. Russell, W. O., and Callaway, C. P.: Arch. Path. **35**:546, 1943.

color. The collodion was removed from the sections by washing them in absolute alcohol. The sections were then stained with hematoxylin and counterstained with light green. In the finished section the deposits of silver were varying shades of brown, the nuclei of the cells were blue and the cytoplasm was green. The amount of silver precipitated in the section varied directly with the degree of activity of alkaline phosphatase present in different foci and for general purposes of comparison may be taken as evidence of the quantity of the enzyme in those foci. Good results were obtained in demonstrating alkaline phosphatase in the tissues in all instances.

## RESULTS

*Lesion in Right Gluteal Muscle.*—All of the animals in the three different groups showed essentially the same type of pathologic change in the muscle at the site of injection of the tubercle bacilli. In all instances there was an abscess cavity filled with a moderately cellular exudate showing large foci of caseous necrosis. The exudate was composed of large numbers of polymorphonuclear leukocytes with scattered macrophages and lymphocytes. There was a broad zone of reaction in the surrounding muscle composed of tuberculous granulation tissue with active proliferation of fibroblasts and deposition of moderate amounts of collagen. It was most unusual to find a giant cell of the Langhans type, and no isolated discrete tubercles were seen.

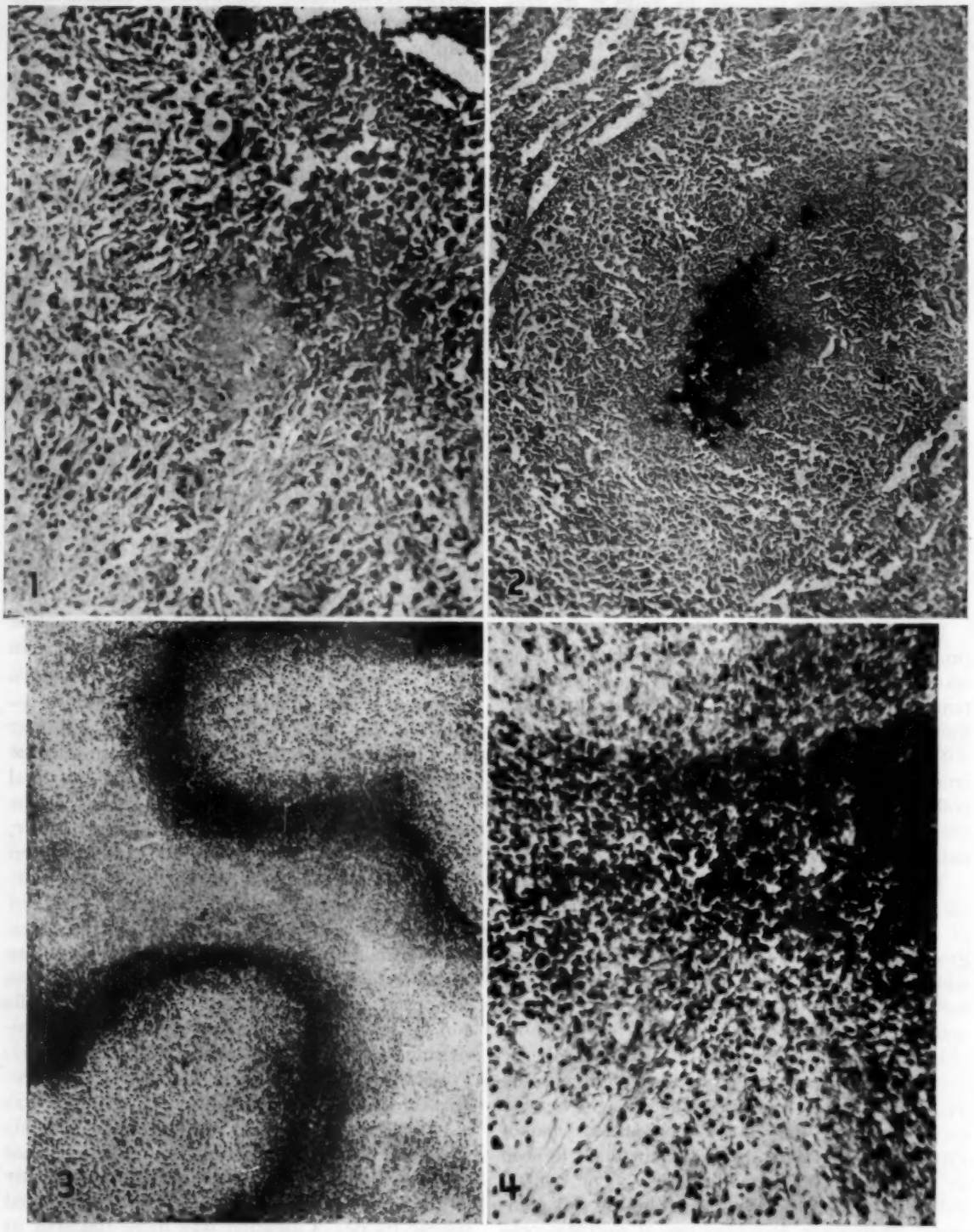
In the deficient animals the zone of fibroblastic proliferation and tuberculous granulation tissue was slightly less prominent and frequently contained small foci of necrosis infiltrated with polymorphonuclear leukocytes. As to sections stained with Masson's trichrome stain for collagen, those from the deficient animals disclosed slightly less collagen in the zone of fibroblastic proliferation than was seen in the sections from the control animals of either group.

The sections stained for alkaline phosphatase showed a constant and characteristic distribution of the enzyme in all the animals of the three groups. The alkaline phosphatase was concentrated in a broad zone at the periphery of the cellular exudate in the abscess cavity; in this zone were included large numbers of polymorphonuclear leukocytes at the junction of the necrosis and the surrounding granulation tissue (fig. 3). The alkaline phosphatase was heavily concentrated in the polymorphonuclear leukocytes, and a small amount of the enzyme was seen in the spaces between the cells. The central half of the zone of alkaline phosphatase was composed of a dense accumulation of necrotic cells with pyknotic nuclei in a caseous-appearing type of necrosis (fig. 4). The alkaline phosphatase in this part of the zone was evenly distributed through the liquefied material. Little or no alkaline phosphatase was visible in the necrotic caseous material in the center of the abscess. In the small abscess cavities occasionally seen in the wall of the large abscess, the enzyme was heavily concentrated, the material contained in these cavities being composed mainly of disintegrating polymorphonuclear leukocytes giving a positive reaction for alkaline phosphatase.

*Lymph Nodes from Right Inguinal Region.*—Microscopic sections showed acute tuberculous inflammation, with large foci of caseation in all sections with complete or nearly complete destruction of the lymph node. Giant cells and the formation of discrete tubercles were infrequently seen, and there was a remarkable tendency for caseous necrosis to be widespread. Large numbers of polymorphonuclear leukocytes were seen at the periphery of the area of caseation among the epithelioid cells and tuberculous granulation tissue. There was no deposition of fibrous tissue around any of the lesions.

Alkaline phosphatase was demonstrated at the periphery of the area of caseation and in the zone of polymorphonuclear leukocytes seen at the junction of the caseous material and the surrounding tuberculous granulation tissue. The polymorphonuclear leukocytes that gave a positive reaction for alkaline phosphatase comprised approximately one third of the zone of alkaline phosphatase. The enzyme was not present in the centers of the larger foci of caseous necrosis.

*Spleen.*—The tuberculous inflammation produced in the spleen was qualitatively different from that in the sections from the abscess in the gluteal muscle and that in the right inguinal lymph nodes. In contrast to the lesions in the inguinal lymph nodes and in the gluteal muscle, where there was acute tuberculous inflammation with infiltration by large numbers of polymorphonuclear leukocytes and a paucity of giant cells and epithelioid cells, the lesions in the spleen showed the more usually observed tissue response to tubercle bacilli, with well defined tubercles composed of large numbers of epithelioid cells and an occasional Langhans giant cell and presenting caseous necrosis of their centers (fig. 1). Polymorphonuclear leukocytes were but infrequently seen in these lesions. A difference was noted between the lesions in the deficient animals and those in the control animals. In the deficient animals the individual lesions were remarkably larger and less well demarcated from the normal splenic tissue. Caseation was more frequent in the large conglomerate tubercles of the deficient animals than in the lesions of the control animals. When sections were stained with Masson's trichrome stain, the tuberculous lesions in the deficient animals showed less collagen between




FIGURES 1 TO 4

the fibroblasts and the epithelioid cells at their peripheries than did those in control animals.

Alkaline phosphatase was demonstrated in lesions having well developed foci of caseation, and occasionally small amounts of the enzyme were demonstrated in macrophages in the splenic sinuses of uninvolved tissue at some distance from the lesions. In the smallest tubercles, with beginning caseation or only a slight amount of caseous material, alkaline phosphatase was never noted (fig. 1), but in all other lesions showing a moderate amount of caseous material there was an abundance of the enzyme (fig. 2). Between the zone of phosphatase and the surrounding tuberculous granulation tissue there was a small zone of caseation that contained no phosphatase (fig. 2). If the focus of caseation was sufficiently large, the alkaline phosphatase disappeared from the center of the necrotic material and was seen as a heavy brownish black coloration at the periphery of the area of caseation. As determined by examination of many tubercles showing varying degrees of caseation, alkaline phosphatase did not appear coincident with caseous material but appeared shortly after the first changes of necrosis. Following this initial latent period in the earliest stage of caseation, alkaline phosphatase appeared in the focus of caseation as a diffuse brownish coloration of the necrotic cells.

*Liver.*—The histologic study of the liver confirmed the impression made in the gross examination of this organ at necropsy that the caseous foci were remarkably larger in the deficient animals than in either of the control groups. No difference in amount of caseation was noted in the two control groups of animals. In both control groups of animals discrete cellular tubercles without central caseation, composed of collections of epithelioid cells and lymphocytes, were scattered throughout the liver (fig. 6). Giant cells of the Langhans type were rarely seen. In the deficient animals, on the other hand, the liver contained many more tubercles per unit of area, and the tubercles were in most instances larger than those in the control animals (fig. 5). Caseous necrosis of the centers of the larger conglomerate tubercles was frequent, and Langhans giant cells were infrequent, although more of these cells were present than in the control animals. A moderate amount of fatty metamorphosis of the liver was noted in the deficient animals, but only occasionally did an animal in the control groups show this pathologic change. No collagen was demonstrable at the peripheries of the lesions in either the control or the deficient animals in sections stained with Masson's trichrome stain.

As to sections stained for alkaline phosphatase, only those from the deficient animals showed significant amounts of the enzyme, which in all instances was in the central part of the focus of caseation in the larger conglomerate tubercles. The enzyme was not seen in the smallest tubercles with beginning caseation or only a slight amount of caseous material. Occasionally, however, a macrophage in a cellular tubercle contained a few brownish granules of alkaline phosphatase. The amount of alkaline phosphatase seen in the caseous foci of the deficient animals was small due to the infrequency of central caseation of the tubercles. Since caseation was not noted in the tubercles in the liver of any control animal, it was impossible to compare scorbutic and nonscorbutic animals with reference to the alkaline phosphatase in this organ.

EXPLANATION OF PLATE—FIGS. 1 TO 4

Fig. 1 (deficient animal 5).—Tubercle in a section of spleen showing a small focus of caseous necrosis. Alkaline phosphatase is not present, although the enzyme was present in larger foci of caseation in the same slide. Paraffin section stained for alkaline phosphatase; × 135.

Fig. 2 (deficient animal 5).—Section of spleen. Conglomerate tubercles in spleen with a large central focus of caseous necrosis. Alkaline phosphatase is shown as the dark coloration in the center of the necrosis. Note at the periphery and surrounding the material containing the alkaline phosphatase a zone of caseous necrosis that contains no enzyme. Paraffin section stained for alkaline phosphatase; × 135.

Fig. 3 (control animal 2).—Section taken from the lesion produced in the right gluteal muscle by the injected tubercle bacilli. Note the broad zone of alkaline phosphatase surrounding each focus of caseous necrosis and the disappearance of the enzyme from the central part of each area of caseation. The alkaline phosphatase extends peripherally to the surrounding tuberculous granulation tissue. Compare with figure 2, where the alkaline phosphatase is absent from a small peripheral fringe of the area of caseation. Paraffin section stained for alkaline phosphatase; × 75.

Fig. 4 (control animal 2).—High power magnification of a part of a zone of alkaline phosphatase seen in figure 3. Note the large numbers of polymorphonuclear leukocytes infiltrating the peripheral part of the lesion in the lower half of the field. The polymorphonuclear leukocytes contain alkaline phosphatase as demonstrated by the methods of Gomori,[5] and Takamatsu,[6] and these cells participate in the formation of the broad zone of phosphatase seen. Note a nearly complete disappearance of the enzyme in the necrotic material seen at the upper part of the field, which is central to the zone of alkaline phosphatase. Paraffin section stained for alkaline phosphatase; × 228.

*Kidney.*—Tubercles were found in sections of kidney from 3 of the deficient animals, 2 of the normal control animals and 1 of the inanition control animals. In all instances they were small cellular lesions and did not show caseation.

In the sections of kidney stained for alkaline phosphatase none of the tubercles contained the enzyme. Large amounts of the enzyme were present in the cells of the proximal convoluted tubules in all groups. Comparison of the three groups of animals with respect to the amount of alkaline phosphatase contained in the cells of the proximal convoluted tubules disclosed that there was significantly more alkaline phosphatase in all but 2 of the deficient animals when the sections were compared with sections taken from corresponding control animals.

*Lung.*—Histologic examination of sections of lung showed discrete small cellular tubercles evenly scattered in all parts of the parenchyma in the lungs of all three groups of animals. No caseation was noted in the tubercles in any of the sections, and Langhans giant cells were rarely seen. There were slightly more tubercles in the lungs of the deficient animals than in those of either control group. Sections stained for alkaline phosphatase disclosed no phosphatase in any of the tubercles.

*Heart.*—Study of sections of heart showed tubercles in the hearts of 1 deficient animal, 2 inanition control animals and none of the normal controls. All of the tubercles noted were small cellular lesions without caseation.

*Adrenal Gland.*—None of the sections of adrenal gland contained tubercles except those representing a vitamin-deficient animal. The tubercle observed was a small discrete lesion composed of epithelioid cells without caseation.

## COMMENT

Two types of tuberculous lesions were noted in the guinea pigs in this experiment. The first type was an acute tuberculous inflammation characterized by an acute inflammatory response in the tissue with large numbers of polymorphonuclear leukocytes infiltrating the periphery of the necrotic material (fig. 4). This type of lesion was found in the abscess formed in the gluteal muscle at the site of the injection of the tubercle bacilli and in the lymph nodes in the right inguinal region draining the abscess. The second type of lesion was noted in the liver, the spleen, the lungs, the heart and the kidneys. The tuberculous lesions in these organs demonstrated the more usually observed type of tuberculous inflammation and were characterized by the

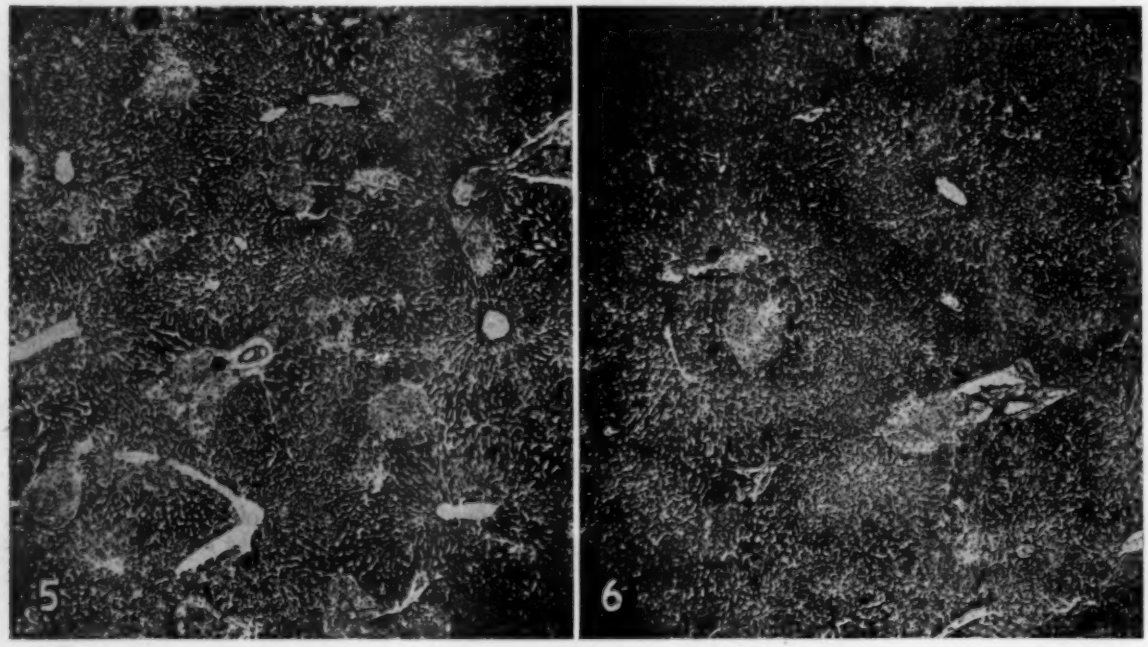



Fig. 5 (deficient animal 1).—Section of liver showing many cellular tubercles scattered throughout. Note the fatty metamorphosis of the liver evidenced by the fine vacuolation of the liver cells. Compare the number of tubercles seen in this section with the number in figure 6. Hematoxylin and eosin; × 75.

Fig. 6 (control animal 1).—Section of liver showing only an occasional tubercle. Compare with figure 5. Hematoxylin and eosin; × 75.

production of discrete tubercles composed of epithelioid calls and frequently showing central caseation and Langhans giant cells (fig. 1). Lesions of this type contained only rare polymorphonuclear leukocytes even though in many instances there were necrosis and caseation of the tissue.

Alkaline phosphatase appeared in both types of lesions if sufficient caseation was present, but the distribution of the enzyme was slightly different in the two types. In the acute type of lesion seen in the gluteal muscle and in the inguinal lymph nodes the alkaline phosphatase was heavily concentrated in the peripheral part of the focus of caseous necrosis and extended even into the zone of infiltrating polymorphonuclear leukocytes at the extreme periphery (fig. 3). Because in guinea pigs polymorphonuclear leukocytes give strongly positive evidence of the presence of alkaline phosphatase,[8] it is reasonable to assume that the zone of alkaline phosphatase seen distal to the periphery of the focus of caseous necrosis resulted from the liberation of the enzyme from the disintegrating polymorphonuclear leukocytes. Gomori[9] has concluded from his studies of alkaline phosphatase in tuberculous lesions of guinea pigs that all of the enzyme appearing in the area of caseous necrosis was carried there by polymorphonuclear leukocytes and liberated when these cells disintegrated. A similar conclusion was reached by Takeuchi and Takamatsu.[10] We do not feel that the results of our experiment entirely support the conclusions of these authors that all of the alkaline phosphatase observed in connection with the process of caseation can be accounted for by disintegrating polymorphonuclear leukocytes. It is readily apparent from our studies that a large part of the alkaline phosphatase seen at the extreme periphery of the zone of phosphatase in the lesions of the gluteal muscle and the inguinal lymph nodes was alkaline phosphatase liberated from the polymorphonuclear leukocytes, but it is difficult to believe that all of the alkaline phosphatase in the focus of caseation was of this source, because the enzyme was present in the same general location at the periphery of the focus of caseation in the lesions of the spleen and the liver, where only a rare polymorphonuclear leukocyte was noted. A basic similarity in the two processes cannot be denied, although each is qualitatively different. It would appear that the same mechanism is concerned with the production of the alkaline phosphatase in each type of lesion and that the polymorphonuclear leukocytes contribute proportionally when present.

It is difficult to explain adequately, however, the source of the alkaline phosphatase not having its origin in polymorphonuclear cells. Judging from the study of tubercles of varying sizes and ages in the sections of spleen and liver, the appearance of alkaline phosphatase is not coincident with necrosis and caseation of the epithelioid cells, there being a brief period in the process when the necrotic cells do not contain the enzyme. Alkaline phosphatase appears first in the center of the focus of caseation surrounded by a narrow zone of caseous material containing no alkaline phosphatase. As the focus of caseation enlarges, the alkaline phosphatase advances peripherally with the caseation but disappears from the necrotic debris in the center, the result being a zone of alkaline phosphatase at the border of the advancing caseation surrounding a phosphatase-free region of caseous material. The absence of alkaline phosphatase in the most recent caseous material suggests that this enzyme is not of autochthonous origin, liberated from the necrotic cells, but probably diffuses into the necrotic tissue from the blood. If this explanation is correct, it is to be assumed that the caseous necrosis for a brief period has no affinity to hold the enzyme but develops that after this brief period since in the larger areas of caseation alkaline phosphatase was always demonstrated. As the line of caseation advances, the necrotic material again loses its affinity for the enzyme or else the alkaline phosphatase does not penetrate the mass and the concentration of the enzyme in the center of the caseous material is not maintained. Gomori,[9] studying the distribution of alkaline phosphatase in experimental tuberculosis in rabbits, expressed the belief that the alkaline phosphatase appearing in the lesions was derived solely from the blood since polymorphonuclear leukocytes of the rabbit do not contain alkaline phosphatase and therefore the presence of this enzyme in caseating lesions of the rabbit could not be explained on this basis, which he stated adequately explains the origin of the alkaline phosphatase in the tuberculous lesions of guinea pigs.

A deficiency of vitamin C is known to lower the alkaline phosphatase level of the blood and the amounts of this enzyme contained in bone, intestinal mucosa and kidney.[3] It might be expected, therefore, that as a result of the general lowering of alkaline phosphatase in somatic tissues and fluids in consequence of vitamin C deficiency the alkaline phosphatase contained in the caseous lesions of tuberculosis would be

8. Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941.

9. Gomori, G.: Am. J. Path. **19**:197, 1942.

10. Takeuchi, T., and Takamatsu, H.: Tr. Jap. Soc. Path. **30**:127, 1940.

lowered correspondingly. We have been unable, however, to detect any difference in the amount or the distribution of the alkaline phosphatase in the lesions of the deficient animals that could not be accounted for by the effect of the scurvy on the course of the tuberculous disease. It is possible, however, that the method employed was not sufficiently sensitive to detect quantitatively a small difference in the amount of enzyme present. While it is a well established fact that alkaline phosphatase is lowered in certain tissues and in the blood by a deficiency of vitamin C, it does not disappear from these somatic tissues or the blood, and the concentration remaining may be entirely sufficient to provide the enzyme in sufficient quantity to the caseous material having an affinity for it.

The results of our experiment confirm the work of other investigators that scurvy adversely affects the course of experimental tuberculosis.[1] It is significant that in 3 deficient animals sinus tracts developed in the gluteal muscle from the injection of the tubercle bacilli while a sinus tract developed in only 1 animal in both control groups. The most striking difference, however, was noted in the sections of the livers of the deficient animals as compared with the controls. Not only were there more tubercles per unit of area in the liver in the cases of deficiency of vitamin C but the tubercles were larger, and there was occasionally caseation of the tubercles, which was never seen in the tubercles in sections of liver representing the control animals. The greater number of tubercles in the livers of the deficient animals would indicate either that there was a greater dissemination of tubercle bacilli in the animals or that the resistance of the liver to infection was impaired by the deficiency, which would also account for the greater size of the tubercles in this organ. There is some evidence that both of these suppositions may be correct.

As to the point of greater dissemination of the tubercle bacilli, there is evidence from the study of the other organs that there was greater dissemination of tubercle bacilli in the deficient animals, because tubercles were more frequent per unit of area in the sections of lung and spleen representing the deficient animals than in corresponding controls. On the other hand, the large size of the lesions and even their incidence could be attributed to an alteration of the liver due to the vitamin deficiency, because there was proportionally a much greater effect of the deficiency on the lesions produced in the livers of the deficient animals than was noted in the other organs studied. Russell and Callaway,[7] working with guinea pigs, reported that a deficiency of vitamin C produced marked fatty metamorphosis of liver cells and that these cells stored more vital dye than those of animals receiving the vitamin and concluded from this that the lack of vitamin C produced hepatic damage. The results which we have reported here seem to lend support to the observation of these authors that deficiency of vitamin C produces hepatic change, for not only did we note fatty metamorphosis of the liver cells, but we observed that the deficiency apparently lowered the resistance of this organ to tuberculous infection, which was disproportionate to the resistance to the infection seen in the other tissues studied.

Although it has been shown that there is a remarkable species variation in the amount of alkaline phosphatase contained in different somatic tissues and cells, the appearance of the alkaline phosphatase is so characteristic and constant a part of the process of caseation that it may be supposed that the specificity of tissue played, if any, only an insignificant role in the general process. Gomori,[9] studying alkaline phosphatase in areas of tuberculous caseation in rabbits, guinea pigs and man, observed the same general distribution of the enzyme in these three species. For these reasons the results obtained with guinea pigs should be applicable to other animals whose tissues react to tubercle bacilli in the same general way with the formation of epithelioid cells, giant cells and foci of caseation.

## SUMMARY AND CONCLUSIONS

Scurvy produced more extensive tuberculosis in guinea pigs infected with tubercle bacilli than was noted in control animals receiving vitamin C. There was evidence of greater dissemination of the tubercle bacilli in the viscera of the scorbutic animals, and the tuberculous lesions generally showed more extensive caseation. The deposition of fibrous tissue around the tubercles was slightly greater in some organs of control animals receiving vitamin C.

Alkaline phosphatase was studied by histochemical methods in the lesions showing caseation. From the study of tubercles of varying size and stages of development it was concluded that alkaline phosphatase does not appear in areas of caseation simultaneously with the development of necrosis of the cells, because the smallest tubercles with small caseous centers did not contain the enzyme and in larger lesions containing alkaline phosphatase there was a small peripheral zone of necrosis without phosphatase between the necrotic material containing the enzyme and the surrounding inflammatory cells. In the large foci of necrosis, alkaline phosphatase disappeared

from the central mass of caseous material and was noted in a broad zone in the periphery of the advancing caseous necrosis. Exception to this distribution of the alkaline phosphatase in the area of caseous necrosis was noted only when the tuberculous process produced an acute type of inflammatory reaction with infiltrations of large numbers of polymorphonuclear leukocytes containing alkaline phosphatase as demonstarted by the methods of Gomori [5] and Takamatsu,[6] at the periphery of the caseous necrosis. In this type of response the small zone of necrosis without alkaline phosphatase usually seen at the extreme periphery of the area of caseous necrosis was not observed, owing to the presence of the polymorphonuclear leukocytes containing alkaline phosphatase in this area.

These observations indicate that polymorphonuclear leukocytes may in certain instances contribute to the alkaline phosphatase in caseous lesions of guinea pigs but are not the sole source of the enzyme seen in connection with caseation. There is some evidence to indicate that alkaline phosphatase in areas of caseation is not of autochthonous origin but is probably derived from the serum, the enzyme diffusing into the area of caseation.

Scurvy produced no demonstrable change in the distribution and the amount of alkaline phosphatase in the caseous foci that could not be attributed to the greater amount of caseation resulting from the effect of the scurvy on the course of the tuberculosis. It is concluded from these observations that if the activity of alkaline phosphatase in caseation is a factor concerned with tissue resistance to tuberculosis, its role is insignificant and apparently not influenced by vitamin C.

# A HISTOCHEMICAL STUDY OF THE EFFECT OF SCURVY ON THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE KIDNEYS OF GUINEA PIGS

WILLIAM O. RUSSELL, M.D.
ST. LOUIS
LIEUTENANT ERNEST T. ROUSE
MEDICAL CORPS, ARMY OF THE UNITED STATES
AND
JAMES ALLAN READ, M.D.
ST. LOUIS

In an experiment in which the effects of a deficiency of ascorbic acid on the alkaline phosphatase in caseating lesions of experimental tuberculosis were studied by histochemical methods, it was noticed that the cells of the proximal convoluted tubules of the kidneys of the scorbutic animals contained less alkaline phosphatase than was observed in the corresponding cells of the control animals.[1] Because in this experiment all of the animals were infected with tubercle bacilli and had active lesions of tuberculosis, a second experiment was made in which the guinea pigs were without experimental tuberculosis and better controlled for this particular observation. In the following communication we report the distribution and the amounts of alkaline phosphatase in the kidneys of the animals in these two experiments and in the mucosa of the small intestine in the second experiment, as determined by histochemical methods.

## EXPERIMENTAL PROCEDURE

In each experiment scurvy was produced in the guinea pigs by feeding a ration known as rabbit chow checkers. This is a commercially prepared food for rabbits sold by Purina Mills Company of St. Louis. The types of food materials used in its preparation, together with its complete chemical analysis, have been published elsewhere.[2] The laboratory department of the Purina Mills Company has been unable to identify any ascorbic acid in this ration by chemical analysis, and if guinea pigs are fed rabbit chow checkers exclusively, characteristic signs of scurvy develop and the animals die in from twenty-one to twenty-five days.

In the first experiment the control animals were fed lettuce ad libitum as a source of ascorbic acid. The inanition effect produced by the scurvy was controlled by a second control group of animals, which received lettuce ad libitum but from which the rabbit chow was withheld and the animals selectively starved so that they lost approximately the same amount of weight as the animals not receiving lettuce. Every guinea pig in this experiment was inoculated in the right hindleg with 0.5 mg. of virulent human tubercle bacilli of the H-160 strain of Corper one day after being placed on its experimental diet. The animals deficient in vitamin C were fed lettuce in two three day periods during the experiment in order to prolong their lives and so allow adequate time for the development of the characteristic lesions of tuberculosis, because guinea pigs will survive at best not more than twenty-five days when fed only rabbit chow. All of the animals were killed forty-three days after being placed on the experimental diets, those deficient in vitamin C following a continuous eleven day period of deficiency. The vitamin C–deficient guinea pigs when killed were in extremely poor condition as a result of the deficiency of ascorbic acid plus the effect of the tuberculous infection. Two of the guinea pigs were found dead several days before the experiment was terminated. These animals were not included in the study because it was felt that postmortem autolysis of the tissues might have seriously altered the alkaline phospatase present.

In the second experiment the deficient, control and inanition control groups of animals were arranged as in the first experiment but the control groups received orally 0.3 mg. of ascorbic acid per day, dissolved in water. Ascorbic acid rather than lettuce was used in this experiment in order to avoid giving the "grass juice factor" [3] to the control animals. The animals deficient in vitamin C were kept continuously on the diet deficient in vitamin C, and all of them were killed nineteen days after being placed on the experimental diet. In the guinea pigs receiving no ascorbic acid during this nineteen day period, the clinical signs of scurvy developed, and there was an average loss of 117 Gm. in weight per animal. The normal control animals gained on an average 80 Gm. per animal, and the inanition control animals lost on an average 70 Gm. per animal.

Duplicate blocks of liver, spleen, kidney, adrenal gland and lung were taken in both experiments. Because in the second experiment we were primarily interested in studying the effect of a deficiency of ascorbic acid on the activity of alkaline phosphatase in otherwise normal tissue, the small intestine was included for study since the mucosa is known to con-

From the Department of Pathology, Washington University School of Medicine.

1. Russell, W. O.; Read, J. A., and Rouse, T. E.: Arch. Path., this issue, p. 31.

2. Russell, W. O., and Callaway, C. P.: Arch. Path. **35**:546, 1943.

3. Randle, S. B.; Sober, H. A., and Kohler, G. O.: J. Nutrition **20**:459, 1940.

tain large amounts of alkaline phosphatase. One set of blocks was fixed in Zenker's fluid, and sections were prepared and stained with hematoxylin and eosin. The second set of blocks was fixed in 95 per cent alcohol, and paraffin sections were prepared for the histochemical demonstration of phosphatase as described by Gomori [4] and Takamatsu.[5] The precise technic followed in the preparation of the sections has been published elsewhere.[1]

In sections prepared by the methods of Gomori and Takamatsu the site of alkaline phosphatase activity in the tissue is marked by a brownish black precipitate of silver that varies directly with the degree of activity of the alkaline phosphatase present in the different regions and for purposes of comparison may be taken as evidence of the relative quantity of the enzyme present. A counterstain of hematoxylin with light green was used, and in the finished section the deposits of silver were varying shades of brown, the nuclei of the cells were blue and the cytoplasm was green. Because the demonstration of alkaline phosphatase in tissue sections is such a complicated procedure, involving many solutions and staining times, special precautions were exercised to avoid possible technical errors. When slides were to be compared individually—for example, those representing deficient animal 1, control animal 1 and inanition control animal 1—the three slides were stained together, side by side, in the same staining basket, so that each slide was exposed to the solution for exactly the same length of time. Good technical results were obtained in demonstrating alkaline phosphatase in the tissues in all instances.

TABLE 1.—*Alkaline Phosphatase in the Kidney (First Experiment, with Experimental Tuberculosis)*

| | Guinea Pigs Deficient in Vitamin C | | Normal Control Animals | | Inanition Control Animals | |
|---|---|---|---|---|---|---|
| Animal | Weight of Animal, Gm. | Phosphatase Activity in Kidney * | Weight of Animal, Gm. | Phosphatase Activity in Kidney | Weight of Animal, Gm. | Phosphatase Activity in Kidney |
| 1 | 257 | ++ | 304 | ++ | 292 | + |
| 2 | 308 | + | 214 | +++ | 298 | +++ |
| 3 | 358 | + | 299 | ++++ | 379 | +++ |
| 4 | 315 | + | 398 | +++ | 284 | +++ |
| 5 | 367 | + | 285 | +++ | 337 | +++ |
| 6 | 410 | + | 398 | +++ | 294 | ++ |
| 7 | 297 | + | 337 | +++ | 327 | ++ |
| 8 | 280 | + | 316 | + | 357 | + |
| 9 | ... | Died | ... | Died | 336 | +++ |
| 10 | 310 | + | 280 | ++ | 315 | ++ |

* Four plus represents the maximum amount of activity observed; one plus the minimum.

## RESULTS

The individual results for the animals in the two experiments are recorded in tables 1 and 2. In estimating the amount of alkaline phosphatase present in a section a system of pluses was used. Four plus was used to indicate the maximal amount of alkaline phosphatase activity observed in any one section and one plus the smallest amount.

In both experiments the sections of kidneys showed a significant decrease in alkaline phosphatase activity in the cells of the proximal convoluted tubules in the scorbutic animals. The study of the mucosa of the small intestine in the second experiment showed a decrease in alkaline phosphatase activity in only a small number of the scorbutic animals. There was no change in activity of alkaline phosphatase in the other tissues studied that could be attributed to deficiency of ascorbic acid. Because it has been demonstrated that the endothelium of the blood vessels is a site of alkaline phosphatase and that hemorrhages resulting from rupture of blood vessels are a prominent manifestation of scurvy, careful attention was given to the alkaline phosphatase in the endothelium of blood vessels. In none of the organs studied, however, was there any observable difference in the alkaline phosphatase of the blood vessels in the three groups of animals that could be attributed to deficiency of ascorbic acid. Because the amount of alkaline phosphatase that can be demonstrated in the endothelium of blood vessels of guinea pigs is insignificantly small as compared with the amount in such tissues as kidney and the mucosa of the small intestine, where large quantities of the enzyme are concentrated, it is possible that small changes in the amounts of the enzyme in the blood vessels could not be detected by the histochemical method employed.

As observed in tissue sections by the Gomori [4] and Takamatsu methods,[5] the cells of the proximal convoluted tubules of the kidney contain large amounts of alkaline phosphatase, which appears as a smudged light brown to black coloring of the cells. The enzyme is most heavily deposited on the free margin of each cell and in the brush border. Traces of alkaline phosphatase are occasionally seen in the epithelium of Bowman's capsule,[6] and in this experiment with guinea pigs we found significant amounts of alkaline phosphatase in the cells of the distal convoluted tubules and occasionally in the cells of the loops of Henle, although the activity of the enzyme in the latter groups of cells is considerably less than that in the cells of the proximal convoluted tubules.

The sections of kidneys from the scorbutic guinea pigs showed the same general pattern of distribution of alkaline phosphatase as was noted in those from the control groups but the intensity of coloration was notably less in the cells of the deficient animals than in those of the control animals. This difference in coloration was so manifest that in most instances a section

4. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**: 23, 1939.

5. Takamatsu, H.: Tr. Jap. Path. Soc. **29**:492, 1939.

6. Kabat, E. A. and Furth, J.: Am. J. Path. **17**: 303, 1941.

of kidney from a scorbutic animal could be distinguished from a section representing a control animal by holding the two slides to the light for comparison (figs. 1 and 2). Microscopic examination of the sections of kidneys confirmed the macroscopic impression that the sections representing the scorbutic animals contained notably less alkaline phosphatase than those representing the control animals (figs. 3 and 4). The brown coloration of the cells of the proximal convoluted tubules, the loops of Henle and the distal convoluted tubules indicating the site of activity of alkaline phosphatase was considerably lighter in the scorbutic than in the nonscorbutic animals. In many instances the cells of the convoluted tubules of the nonscorbutic animals were a deep brown color and in some areas black at the brush border (fig. 3). The cells of the convoluted tubules showing activity of alkaline phosphatase in the scorbutic animals were remarkably lighter colored (fig. 4) when compared with those of the nonscorbutic animals, for only occasionally were there observed the heavy black-brown deposits in the free margin and brush border of the cells of the convoluted tubules that were so conspicuous in the nonscorbutic animals.

The results of the first experiment (table 1) indicate a significant decrease of alkaline phosphatase activity in the kidneys of 7 of the deficient animals. There was no demonstrable difference in alkaline phosphatase activity in the kidneys of 2 scorbutic animals when the sections were compared with their corresponding control sections.

The results of the second experiment (table 2) verified the original observation in the first experiment that scurvy decreases the amount of alkaline phosphatase in the kidneys. In this experiment 9 of the scorbutic animals showed less activity of alkaline phosphatase in the kidneys than their corresponding control. There was no detectable difference in activity of alkaline phosphatase in the kidney of 1 scorbutic animal (7) when it was compared with its corresponding normal control, but the inanition control animal showed slightly more alkaline phosphatase. In both experiments the cells of the proximal convoluted tubules where the change in activity of alkaline phosphatase was noted showed no demonstrable pathologic change in sections prepared from the blocks fixed in Zenker's fluid and stained with hematoxylin and eosin.

No consistently significant change was noted in the alkaline phosphatase content of the mucosa of the small intestine in the scorbutic and nonscorbutic animals in the second experiment. Three of the scorbutic animals showed less activity of alkaline phosphatase in the mucosa than corresponding control animals, but in 3 of the scorbutic animals there was more activity of alkaline phosphatase than in the control animals. Between scorbutic animal 6 (table 2) and the corresponding controls no difference could be detected.

TABLE 2.—*Alkaline Phosphatase in the Kidney and the Mucosa of the Small Intestine (Second Experiment, Without Experimental Tuberculosis)*

| | Guinea Pigs Deficient in Vitamin C | | | Normal Control Animals | | | Inanition Control Animals | | |
|---|---|---|---|---|---|---|---|---|---|
| Animal | Weight of Animal, Gm. | Phosphatase Activity in Kidney * | Phosphatase Activity in Small Intestine | Weight of Animal, Gm. | Phosphatase Activity in Kidney | Phosphatase Activity in Small Intestine | Weight of Animal, Gm. | Phosphatase Activity in Kidney | Phosphatase Activity in Small Intestine |
| 1 | 525 | ++ | ++ | 660 | +++ | ++ | 578 | +++ | +++ |
| 2 | 656 | ++ | ++ | 625 | ++++ | +++ | 520 | +++ | +++ |
| 3 | 650 | ++ | + | 740 | +++ | + | 523 | +++ | +++ |
| 4 | 913 | ++ | ++ | 622 | +++ | + | 580 | ++++ | ++ |
| 5 | 267 | + | + | 650 | +++ | ++++ | 300 | ++++ | ++ |
| 6 | 460 | ++ | + | 613 | +++ | + | 585 | ++ | + |
| 7 | 229 | ++ | +++ | 700 | ++ | + | 525 | ++++ | + |
| 8 | 508 | ++ | ++ | 730 | +++ | + | 635 | Died | |
| 9 | 580 | + | + | 785 | ++ | + | 375 | Died | |
| 10 | 290 | + | + | 370 | +++ | +++ | 485 | Died | |
| 11 | 387 | ++ | + | 500 | Died | | | | |

* Four plus represents the maximum amount of activity observed; one plus, the minimum.

## COMMENT

The results of our experiments are in essential agreement with the findings of Harrer and King,[7] who determined quantitatively by chemical analysis esterase and alkaline phosphatase in brain, kidney, liver and intestinal mucosa of scorbutic guinea pigs. They reported a "moderate decrease" in alkaline phosphatase in the kidney for the scorbutic animals, which had an average value of 322, compared with an average value of 528 for the control animals. Results were expressed as milligrams of phosphorus hydrolyzed per milligram of dry weight of tissue extract. The decrease in alkaline phosphatase in the mucosa of the small intestine was less than that observed in the kidney. As determined for the intestinal mucosa there was an average

7. Harrer, C. J., and King, C. G.: J. Biol. Chem. **138**:111, 1941.

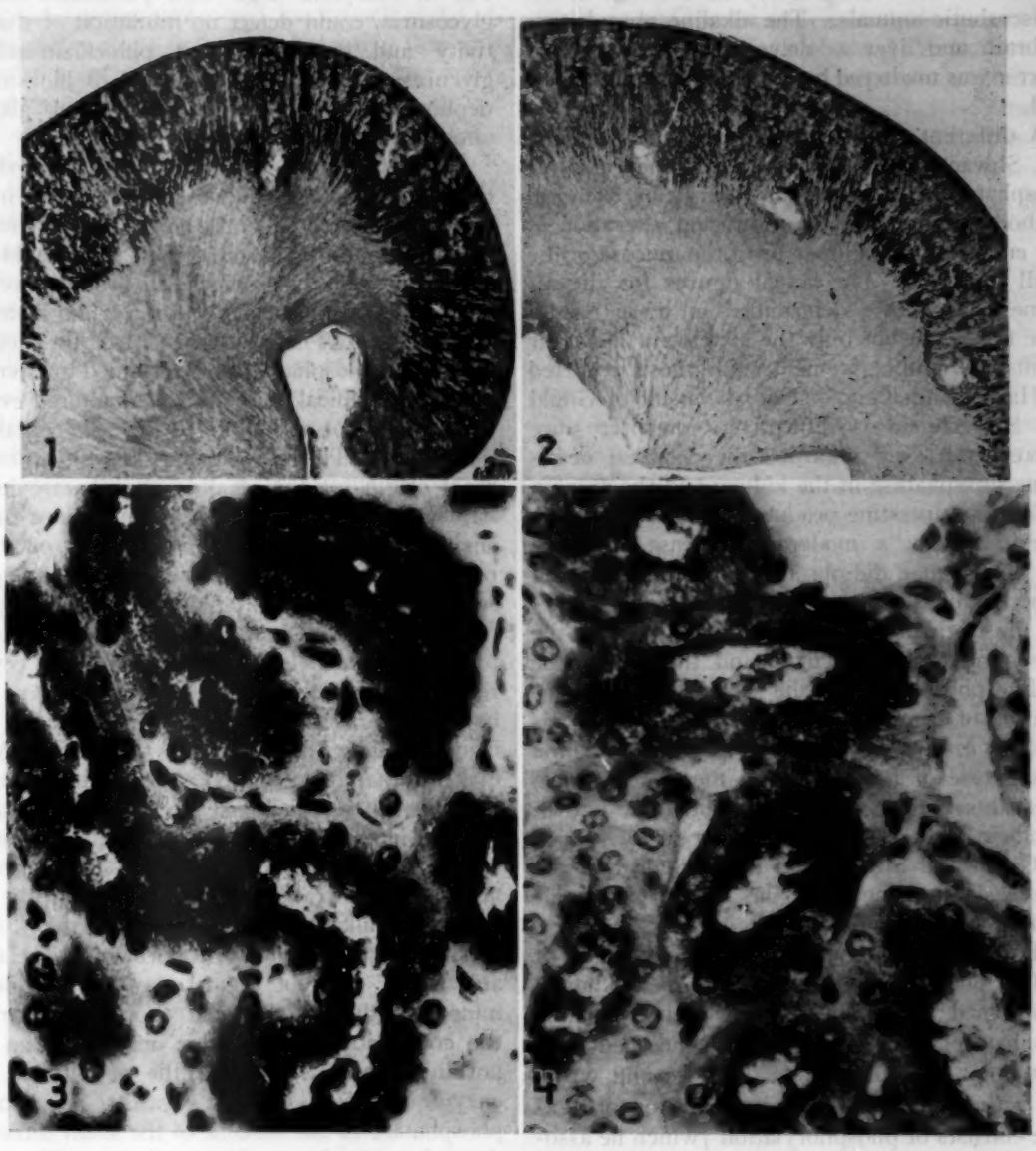



Fig. 1 (control animal 3, second experiment).—Section of a kidney showing heavy deposits of alkaline phosphatase in the cortex, appearing as a black coloration of the renal cells. There is no alkaline phosphatase in the medullary part of the kidney. Paraffin section stained for alkaline phosphatase; × 6.5.

Fig. 2 (deficient animal 3, second experiment).—Section of a kidney showing scattered deposits of alkaline phosphatase in the cortex. Compare with figure 1. The sections in figures 1 and 2 were processed and photographed under identical conditions. Paraffin section stained for alkaline phosphatase; × 6.5.

Fig. 3 (control animal 3).—Higher magnification of a part of the section shown in figure 1. Alkaline phosphatase is shown in the cells of the proximal convoluted tubules and is most heavily concentrated at the free margin of each cell. Paraffin section stained for alkaline phosphatase; × 570.

Fig. 4 (deficient animal 3).—High magnification of a part of the section shown in figure 2. The cells of the proximal convoluted tubules contain small amounts of alkaline phosphatase, most heavily concentrated at the free margin. Compare with figure 3. Figures 3 and 4 were each exposed differently in order to attain the best photographic result. Despite the difference in exposure, the two pictures actually show less difference in the intensity of coloration of the section than was present. Paraffin section stained for alkaline phosphatase; × 570.

value of 461 mg. in the scorbutic animals, compared with an average value of 574 mg. in the nonscorbutic animals. The alkaline phosphatase in brain and liver as determined by King and Harrer was unaltered by the deficiency of ascorbic acid.

A different conclusion was reached by Gould and Shwachman,[8] who also determined alkaline phosphatase in experimental scurvy by chemical methods but reported no significant alteration of this enzyme in the kidney or in the mucosa of the small intestine. The actual figures for the alkaline phosphatase determined in these tissues were not given but only the statement that their results were in agreement with those reported by Harrer and King.[7] This statement of Gould and Shwachman is difficult to interpret since Harrer and King regarded the decrease of alkaline phosphatase in the kidney and the mucosa of the small intestine produced by scurvy in their experiment as "a moderate decrease." Gould and Shwachman did observe, however, a striking reduction of alkaline phosphatase in the bones and the teeth in acute scurvy that paralleled the development of the scurvy and the fall in the alkaline phosphatase of the serum. The administration of ascorbic acid to the deficient animals resulted in a concomitant rise in the alkaline phosphatase of the blood and the bones. This observation when considered with the known histologic changes produced in bone by scurvy led Gould and Shwachman to conclude that the osteoblasts in bone were the source of the alkaline phosphatase in serum.

The specific function of the rich content of alkaline phosphatase in the kidney is unknown. Lundsgaard[9] in 1933 suggested that alkaline phosphatase in the kidney was concerned with the tubular reabsorption of dextrose and wrote "the active step in tubular reabsorption of dextrose consists of phosphorylation [which he associated with the large amounts of the enzyme present in renal tissue] and the effect of phlorhizin is to block phosphorylation." Much evidence, however, has been presented subsequently to discredit this idea of Lundsgaard that phosphorylation of dextrose is blocked by the action of phlorhizin inactivating the renal phosphatase.[10] More recently Kritzler and Gutman,[11] studying the activity of alkaline phosphatase in the kidney by chemical and histochemical methods in rats and dogs with phlorhizin-induced glycosuria, could detect no inhibition of that activity and concluded that phlorhizin-induced glycuresis is apparently not due to blocking of dephosphorylation by inactivation of alkaline phosphatase in the renal tubules.

Hepler, Simonds and Gurley[12] have shown that the activity of alkaline phosphatase in the kidney is unaffected by the action of heavy metals such as uranium bichromate and mercury bichloride even when these substances produce necrosis of the cells of the tubules and in certain instances calcification. They observed that the activity of alkaline phosphatase as determined by chemical and histochemical methods was unaltered even if the metallic poison had killed the cells containing the enzyme. This observation suggests an extrarenal origin for the alkaline phosphatase in the kidney since apparently the maintenance of the enzyme in the tissue is not dependent on viable renal cells. This evidence notwithstanding, it seems most unlikely that the high concentration of alkaline phosphatase in the kidney could be totally explained by a special affinity of certain renal cells to pick up and store the enzyme that had been made in other cells in the body and transported to the kidney by the blood. More evidence than this will have to be submitted before it can be safely concluded that the alkaline phosphatase in the kidney is of extrarenal origin.

It is difficult to explain the physiologic mechanism involved and the significance of the decrease in alkaline phosphatase in the kidney produced by deficiency of ascorbic acid. Harrer and King[7] did not regard the decrease determined by them as marked enough to warrant the conclusion that ascorbic acid plays an important role in regulating the activity of this enzyme in that tissue. In regard to the alkaline phosphatase in the mucosa of the small intestine, there is even less evidence that ascorbic acid is concerned with its activity since in our experiment, in which the enzyme was determined with chemical methods,[7] scurvy produced little or no change in the activity of the enzyme in this tissue. Russell and Callaway[2] reported increased deposits of trypan blue in the cells of the proximal convoluted tubules (site of greatest activity of alkaline phosphatase) of the kidneys of guinea pigs deficient in ascorbic acid without a demonstrable histologic change. This observation was interpreted by them as indicating some altered physiologic function of the cells storing the dye, since trypan blue is known to have an affinity for all diseased cells and necrotic tissue. It is

8. Gould, B. S., and Shwachman, H.: Am. J. Physiol. **135**:485, 1942.

9. Lundsgaard, E.: Biochem. Ztschr. **264**:209, 1933.

10. Lambrechts, A.: Arch. internat. de physiol. (supp. 1) **44**: 1, 1937. Walker, A. M., and Hudson, C. L.: Am. J. Physiol. **118**:130, 1937.

11. Kritzler, R. A., and Gutman, A. B.: Am. J. Physiol. **134**:94, 1941.

12. Hepler, O. E.; Simonds, J. P., and Gurley, H.: Proc. Soc. Exper. Biol. & Med. **44**:221, 1940.

possible that the altered physiologic function of the kidneys as manifested by the increased deposits of trypan blue noted by Russell and Callaway is the same pathologic alteration that produced the decrease in alkaline phosphatase activity observed in our experiments, since the change was noted in the cells of the proximal convoluted tubules in each instance. It is impossible to say from the available evidence whether the altered function of the renal cells, as suggested by Russell and Callaway, causes these cells to take up less alkaline phosphatase, assuming that alkaline phosphatase in the kidney is of extrarenal origin, or whether alkaline phosphatase is not so abundantly produced by the renal cells when the animal is deficient in ascorbic acid, which is to assume that the enzyme is of renal origin. Further investigation will be needed to elucidate this point, particularly in regard to the formation and function of alkaline phosphatase in the mammalian kidney.

## SUMMARY

The distribution of alkaline phosphatase in the kidneys, the liver, the spleen, the lungs, the adrenal glands and the mucosa of the small intestine has been studied by the histochemical methods of Gomori and Takamatsu in scorbutic guinea pigs. Sections of the kidneys from the scorbutic animals in two separate experiments showed less activity of alkaline phosphatase in the cells of the proximal convoluted tubules than sections from the kidneys of the corresponding control animals. No significant alteration in activity of alkaline phosphatase was noted in the other tissues studied. It was not possible to correlate the decreasel activity of alkaline phosphatase in the kidney with any known physiologic function of that organ. The alkaline phosphatase in the intestinal mucosa was determined in only one of the experiments and the results in this experiment indicated that ascorbic acid had little or no influence on activity of alkaline phosphatase in this tissue. The results of our experiments confirm those of other investigators who have determined by chemical methods that experimental scurvy produces a moderate decrease in the alkaline phosphatase in the kidney.

# SPONTANEOUS ARTERIOSCLEROSIS IN CHICKENS

D. V. DAUBER, M.D.
CHICAGO

Of all animals, birds have arteriosclerosis most closely resembling human arteriosclerosis.[1] For this reason the experimental production of arteriosclerosis in birds was undertaken in the laboratory of the cardiovascular department of Michael Reese Hospital. It was reasoned that it would be instructive to reproduce the disease in an animal which is naturally subject to the same type of vascular changes as man. The fact that the chick, like man, is omnivorous would give added significance to the results.

After the production of arteriosclerosis in chicks by cholesterol feeding,[2] it became important to know how closely the experimental lesions reproduced the spontaneous pathologic process. Avian arteriosclerosis has been extensively studied by Fox,[3] and vascular disease in chickens has been described,[4] but the absence of any large series of observations on domestic chickens in this country made it desirable to make such observations for comparison with those on experimental lesions.

## MATERIAL AND METHODS

The material consisted of the aortas of "old roosters" and "fowl" obtained during evisceration at a commercial plant. Material was collected on three different days when large numbers of chickens were being prepared for canning. In the first collection the aortas of 79 hens were obtained. On a second date the aortas of 59 hens were secured and on a third the aortas of 53 roosters. While the exact age of the birds could not be known, the commercial "old roosters" are over 1 year of age, and "fowl" in the trade definition means hens 1 to 2 or more years old. The chickens were of many varieties and sizes. They had been collected from farms in Iowa, shipped to Chicago, killed, quick-frozen for days or weeks (in the case of the roosters, for five months) and later thawed and cleaned for canning. At this stage evisceration was performed and the aortas secured. When possible, the entire aorta and the heart were removed in one piece. However, the technic of evisceration often leaves only a portion of the aorta or the aorta in two or more segments. Statistical studies on this material, which encompasses many unassessable variables, would not be valid. The variables which cannot be evaluated are (1) the uncertain age, (2) the mixture of varieties and (3) the feed, of which the cholesterol content is unknown. About several other factors there is no uncertainty, namely (1) the sex, (2) the fact that the chicks were over 1 year of age and (3) the fact that they were free of tuberculosis, for which the government inspected them. The period of freezing did not seem to damage the aortas although a number were discolored by decomposed blood pigments.

The aortas were opened and examined a few hours after removal. They were then fixed in formaldehyde U.S.P. diluted 1 to 4. Representative lesions were selected for histologic examination. Sections were prepared from paraffin blocks and routinely stained with iron-hematoxylin. In addition, the orcein elastic stain and the Giemsa stain were done on typical lesions. Many aortas were stained in toto with sudan IV in order to observe the macroscopic distribution of fat in the intima. The microscopic localization of fat was studied by means of fat stains of frozen sections.

## RESULTS

Of the aortas from the 53 roosters, 24, or 45 per cent, had gross intimal arteriosclerosis. The total number of aortas from hens was 138; of these, 57, or 41 per cent, showed macroscopic intimal lesions. The frequency of the disease was thus equal in the two sexes, in contrast to the 9:1 preponderance in male birds reported by Fox.[3]

Staining the whole aorta with sudan IV demonstrated intimal lipid in at least half the hens' aortas which had been classed as not showing arteriosclerosis on direct inspection. If these were included in the number with arteriosclerotic lesions, the percentage of pathologic aortas of hens would exceed that of roosters. Only a few of the roosters' aortas which had been classed as not showing arteriosclerotic lesions revealed lipid with sudan IV staining.

In the rooster the abnormalities are limited to the muscular aorta, i. e., the descending thoracic and the abdominal aorta. The abdominal aorta is by far the commonest site of change.

From the Cardiovascular Department, Michael Reese Hospital. This department is supported in part by the Michael Reese Research Foundation.

This investigation was aided by a grant from the Special Cardiac Research Fund of the Committee on Scientific Research of the American Medical Association.

1. Fox, H., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, chap. 6, p. 153.
2. Dauber, D. V., and Katz, L. N.: Arch. Path. (*a*) **34**:937, 1942; (*b*) **36**:473, 1943.
3. Fox, H.: Bull. New York Acad. Med. **15**:748, 1939; footnote 1.
4. (*a*) Kawamura, R.: Neue Beiträge zur Morphologie und Physiologie der Cholesterinsteatose, Jena, Gustav Fischer, 1927. (*b*) Kesten, H. D.; Meaker, D. R., and Jobling, J. W.: Proc. Soc. Exper. Biol. & Med. **34**:818, 1936. (*c*) Uchiyama, T.: Virchows Arch. f. path. Anat. **277**:642, 1930. (*d*) Yamagiwa, K., and Adachi, O.: Verhandl. d. jap. path. Gesellsch. **4**:55, 1914. (*e*) Dauber and Katz.[2b]

In 22 of the 24 cases of sclerosis of the aorta of the rooster the intima of the abdominal aorta was elevated by a longitudinal white or yellow ridgelike thickening of the interrenal region. In the 2 additional cases, as well as in many of the 22 referred to, there were small circumscribed elevated plaques about the orifices of arteries branching from the aorta. In no case was intimal change observed in the elastic aorta, i. e., the ascending aorta and arch, either on simple inspection or after staining with sudan IV.

In the hen the disease of the aorta manifests itself as two distinct types of lesions. First there are nodular and ridgelike intimal elevations of the descending thoracic and the abdominal aorta similar to those observed in the rooster. The smaller, nodular lesions surround orifices of branching arteries, and the longitudinal ridge may be found narrowing the lumen of the interrenal region. As in the rooster, both these lesions vary in color from a semitranslucent bluish white to opaque orange-yellow. Generally, the ridge is smaller and less pronounced than that in the rooster. The nodular elevations frequently stain with sudan IV, in contrast to the ridgelike thickening, which tends to stand out by its failure to take the red stain.

The second type of lesion in the hen's aorta occurs in the elastic aorta (ascending aorta and arch) and is seen in the hen only. It consists of a bright yellow streaky or spotty color change of the intima; the involved area is perfectly smooth, flat and unelevated. When the entire aorta is stained with sudan IV, such yellow streaks take the fat stain. Frequently the intima of the elastic aorta and the brachiocephalic arteries of the hen stains diffusely with sudan IV although no yellow streaking is visible. The intima at the orifices of arteries branching from the muscular aorta likewise may show lipid in the absence of any visible yellow lesion.

The hearts of 11 roosters and several hens were inspected, but no valvular arteriosclerosis and no gross scars were found.

The lesions of the muscular aorta show largely the same microscopic structure in both the rooster and the hen. Essentially the intima is thickened in a varying degree, either diffusely or in a focus, by fibrous tissue. The overlying endothelium is intact. The fibrous tissue comprising the intimal thickening is young and cellular in regions of little proliferation and also at the surfaces of large plaques. On the other hand, the deeper portions of thick plaques are composed of dense, acellular collagenous tissue (fig. 1 *A* and *B*). Hyaline change in the collagen is not uncommon, and in the depths of plaques, close to the media, there are often areas of mucoid degeneration (fig. 1 *A*). In some cases these necrotic zones contain fusiform cholesterol slits and round calcific granules (fig. 1 *C*). In other cases the deeper layers of fibrous plaques contain large pale foam cells (fig. 1 *B* and *D*). Vasa vasorum and extravascular blood cells are often noted in the thickened intima (fig. 1 *H*). Fat stains of frozen sections show varying quantities of lipid in the depths of the thickened intima and also in the adjacent tissue of the media (fig. 1 *E*). In areas of degeneration the fat occurs as extracellular globules and rodlike crystals (fig. 1 *F* and *G*). Close to such areas are fibroblasts containing fat in the cytoplasm at either pole of the nucleus. Such lipid-bearing fibroblasts are also seen in areas of moderate intimal fibrosis which have not undergone degenerative changes. In the rooster these lipid-bearing cells usually lie in the portion of the thickened intima adjacent to the media or in the middle of the plaque. The aorta of the rooster never showed lipid in the intima in the absence of fibrous thickening.

In the hen, it is not uncommon to observe fat in the intima of the muscular aorta in the absence of any fibrosis (fig. 2 *A*). This lipid occurs as granule-like droplets within fusiform cells which are from all appearances fibroblasts. No foam cells have been seen. When both fibrous thickening and lipid accumulation occur in the intima of the hen, the fat may be observed not only in the depths of plaques, as in the rooster, but in the fibroblasts immediately below the endothelium or in the midzone of a plaque (fig. 2 *B* and *D*).

Little reduplication of the internal elastic lamina is noted. Instead, under thick fibrous plaques the internal elastic lamina is often partially destroyed (fig. 1 *B*) and the media compressed. Areas of destruction of the elastic tissue of the media and of degeneration are likewise observed (fig. 1 *B*). However, in no case was medial fibrosis or calcification observed, although Uchiyama [4c] reported this type of arterial disease in chickens. As already noted, in a number of cases in which there was fat in the intima the adjacent media likewise showed fine granular intracellular lipid. In the hen, the quantity of fat in the media is often considerable even when there is little intimal change (fig. 2 *C*). Vasa vasorum are seen in the media as well as in the intima.

The microscopic structure of the orange-yellow streaks in the elastic aorta of the hen is unlike that of the lesions in the muscular aorta. No fibrosis, atheroma, cholesterol slit or calcification is seen. The change consists of an accumulation of lipid within fibroblasts in the intima and the adjacent media (fig. 2 *E*). Extra-

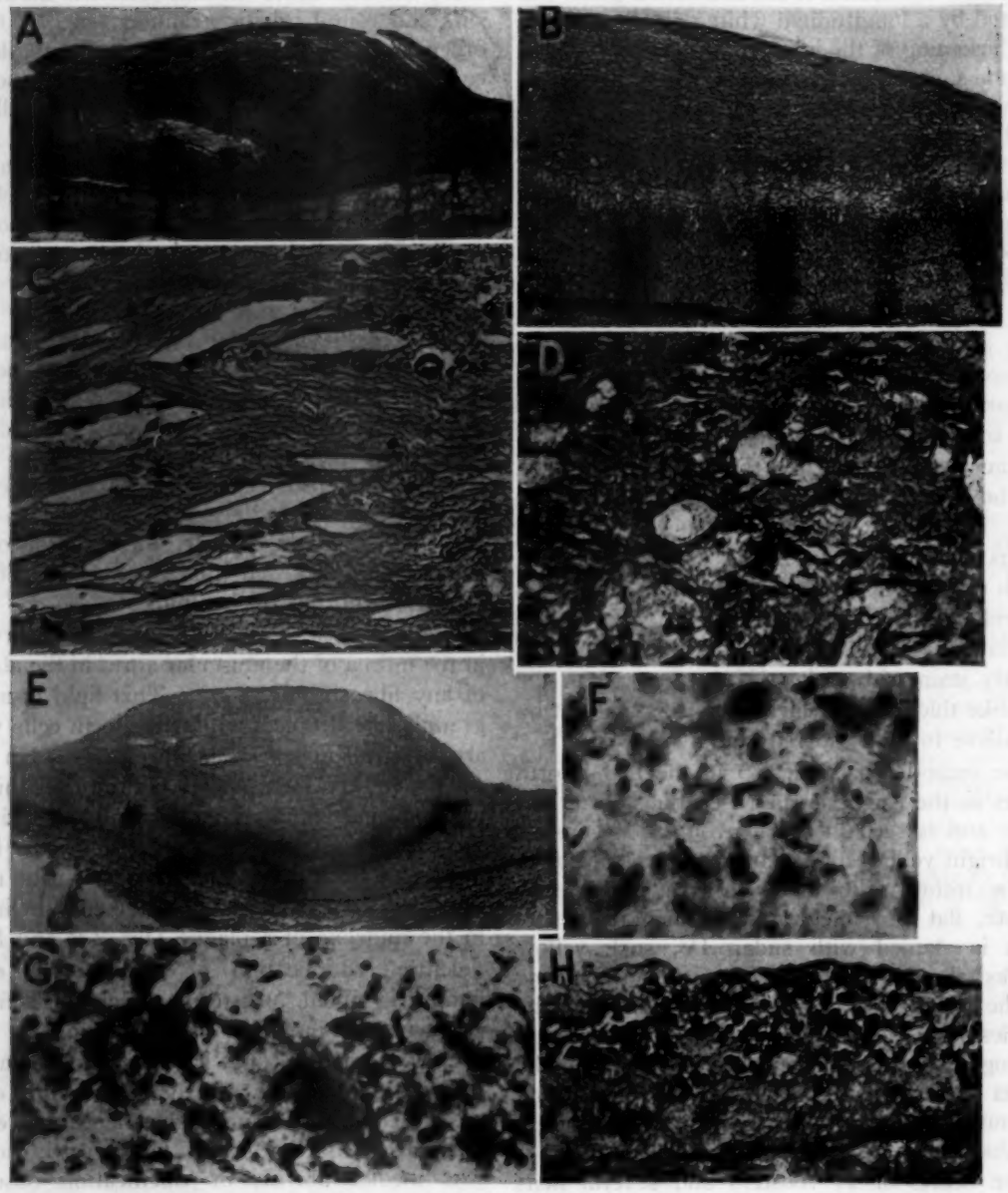



Fig. 1.—Abdominal aorta of a rooster. *A*, transverse section. Note the extensive fibrous intimal thickening. Deep within the fibrous plaque is a light-appearing area of mucoid degeneration. Orcein elastic stain; × 30.

*B*, transverse section through a region of intimal fibrous thickening. Destruction of the internal elastic lamina and of the elastic fibers of the media can be seen. The external elastic lamina remains well defined. At the junction of the thickened intima and the media, numerous large foam cells are clearly seen. Orcein elastic stain; × 56.

*C*, cholesterol clefts and granules of calcific material within the intimal plaque. Orcein elastic stain; × 420.

*D*, foam cells within an area of fibrous thickening of the intima, lying close to the media. Orcein elastic stain; × 420.

*E*, transverse section through an intimal plaque, showing fat deep within the plaque, adjacent to the media. There is also fat within the media, which is compressed under the intimal plaque. Sudan IV stain; frozen section; × 180.

*F* and *G*, a higher magnification of a portion of the area of lipid deep within the intimal plaque in *E*. Note the elongate and fusiform cholesterol crystals as well as the rounded globules of lipid. Sudan IV stain; frozen section; × 600.

*H*, transverse section. Vasa vasorum and extravascular blood cells may be seen within the area of intimal fibrosis. Iron-hematoxylin; × 450.

cellular fat is also present—in the form of fine droplets between elastic fibers. The lipid in the elastic aorta is found in the absence of any fibrous thickening of the intima. No foam cells have been seen.

The coronary arteries studied in sections of heart muscle from the left ventricles of roosters failed to show arteriosclerosis.

Well marked circumferential fibrous thickening of the intima was noted in a small artery seen in the adventitia of the aorta in several sections (fig. 2 *F*).

## COMMENT

The incidence of spontaneous arteriosclerosis involving the aorta in domestic chickens was found to be higher than previously reported for any birds in this country. Fox in his first study [1] found the frequency of arteriosclerosis in ground fowl of the varieties seen in zoologic gardens to be 1.6 per cent. On the basis of later data [2] he reported a higher incidence, namely, 5 per cent. The frequency of arteriosclerosis in birds of all kinds was 13.7 per cent in his later report. In Japan, on the other hand, Yamaguchi

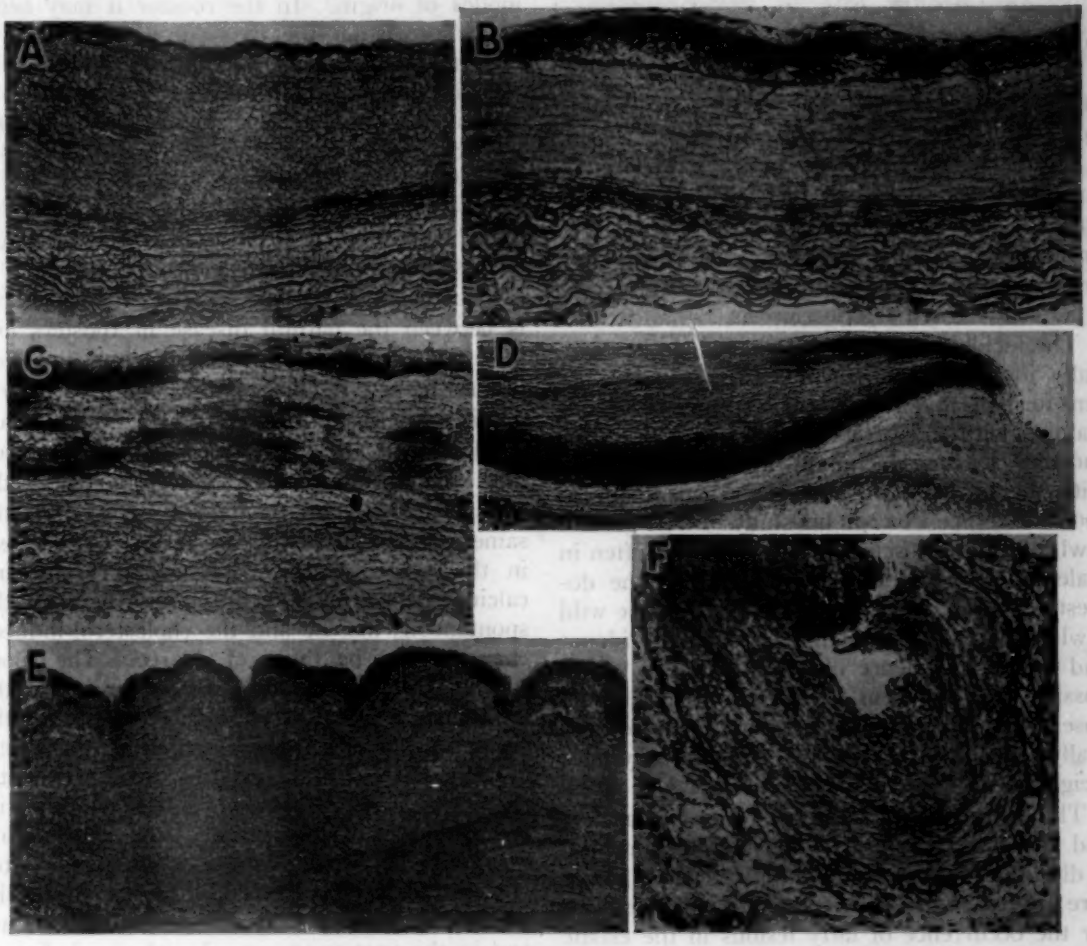



Fig. 2.—*A*, transverse section of the abdominal aorta of a hen, showing the presence of fat within the intima although there is no fibrosis or other change. Sudan IV stain; frozen section, × 132.

*B*, transverse section of the abdominal aorta of a hen, showing slight fibrous thickening of the intima with abundant lipid. Sudan IV stain; frozen section; × 168.

*C*, transverse section of the abdominal aorta of a hen. Fat may be seen in the media as well as in the intima. The fibrous thickening of the intima is slight. Sudan IV stain; frozen section; × 144.

*D*, transverse section of the abdominal aorta of a hen, showing lipid just beneath the endothelium and also deep in the plaque, adjacent to the media. Note how thin the media is under the plaque. Sudan IV stain; frozen section; × 70.

*E*, transverse section of the descending thoracic aorta of a hen, showing lipid within the intima without any other change. Sudan IV stain; frozen section; × 45.

*F*, small artery occurring in the adventitia of the abdominal aorta of a rooster. Note the marked circumferential fibrous thickening of the intima. Orcein elastic stain; × 156.

(quoted by Uchiyama [4c]) found spontaneous coronary arteriosclerosis in 75 per cent of the fowl which he examined. Uchiyama [4c] reported two types of spontaneous arteriosclerosis in domestic chickens. The first, the intimal variety, corresponds to that which I have observed. The second, a primary medial calcification, was not found in any of my series.

The onset of arteriosclerosis in roosters occurs at the age of 5 to 6 months.[2b] In our [2] experience, with healthy laboratory chickens serving as controls, 28 per cent of young roosters between 5 and 9 months of age show spontaneous gross intimal lesions. Kesten and co-workers [4b] found aortic intimal fibrosis in 14 of 25 roosters over 5 months old. The incidence observed in commercial chickens over the age of 1 year is 45 per cent; at the end of the second year of life it may be as high as 75 per cent.[4c] These figures show increasing incidence with advancing age in chickens as in man. On the other hand, the wild ground fowl examined by Fox [3] had been eleven years in captivity on the average, and yet only 5 per cent showed arteriosclerosis.

The incidence of macroscopic change is essentially identical for both sexes, namely, 41 per cent for hens and 45 per cent for roosters. After staining with sudan IV the percentage of aortas showing lesions, including those brought out with the fat stain, is higher for hens than for roosters. Since Fox [1] in his study of wild ground fowl found arteriosclerosis nine times as often in males as in females, it is evident that the domestic chicken differs significantly from the wild fowl with respect to both the general incidence and the sex incidence of arteriosclerosis. It is possible that the hens in the commercial series observed in the present investigation were generally older than the roosters, and this may have weighted the figures for the incidence in hens.

The differences between the lesion of roosters and those of hens are of considerable interest. A difference in location and a difference in structure are noted. In considering the possible basis for the occurrence of fatty lesions in the elastic aorta of the hen and their absence in that of the rooster, the influence of the production of eggs on the cholesterol metabolism of the hen immediately comes to mind. Uchiyama [4c] observed fatty streaks of the elastic aorta in hens dying of peritonitis associated with impaction of eggs. He suggested resorption of egg yolk from the abdominal cavity as the source of the excess cholesterol. Hens have been reported to have a higher level of blood cholesterol during the egg laying period than roosters.

In my search for the earliest change in the course of the development of arteriosclerosis I frequently found simple lipid within fixed intimal cells of the hen's aorta, both those of the elastic and those of the muscular aorta, prior to fibrosis or any other change. In the rooster, lipid alone was never found; the earliest observed change was intimal fibrosis, sometimes with lipid and often without. It may be that lipid is more abundant in the hen and therefore more readily observed. This would make the difference between hens and roosters a quantitative rather than a qualitative one. On the other hand, the observations suggest that spontaneous arteriosclerosis may have two alternative modes of origin. In the rooster it may begin with fibrosis on the basis of inflammation or mechanical stress, with secondary accumulation of lipid. Or it may begin with accumulation of lipid in the intima, as in the hen and in animals with cholesterol-induced lesions, and fibrosis may be secondary. The end result appears to be the same. These observations suggest that there may be multiple processes rather than a single process resulting in arterial intimal lesions.

The question arises as to what similarity there is between the natural disease in roosters and hens and the disease produced by intensive cholesterol feeding in chicks and rabbits. In macroscopic appearance of lesions the two diseases are similar; both are characterized by elevated yellow streaks and plaques. Microscopically the advanced lesions of the two diseases show the same components of fibrous tissue with necrosis in the deeper layers, cholesterol crystals and calcium deposits. The differences between the spontaneous disease and the cholesterol-induced changes may be those of degree. The spontaneous disease in the rooster is limited to the muscular aorta, while cholesterol feeding of the rooster produces extensive change in the elastic aorta as well. In other words, the experimental disease is more widespread, less selective, in its localization. Microscopically, the outstanding difference lies in the role of foam cells. After cholesterol feeding the earliest change is the accumulation of foam cells below the endothelium and in the vasa vasorum. In advanced disease with thick plaques, further accumulation of foam cells occurs subendothelially. In the spontaneous disease, few foam cells are observed, and these are always in the deeper portions of well developed fibrous plaques. It may be that the foam cell is a feature of the early spontaneous disease but that I have not had the good fortune to observe it. In early human arteriosclerosis Leary [5] occasionally noted accumulations of subendothelial foam cells, although the usual examination of human arteriosclerotic vessels does not

5. Leary, T.: Arch. Path. **32**:507, 1941.

disclose them. After forced cholesterol feeding they are present in tremendous numbers and are readily observed, whereas in the ordinary course of the development of arteriosclerosis foam cells may be few and short lived and therefore readily missed. On the other hand, if the observations reported here are accepted as complete and intimal fibrosis is therefore considered the initial change in the rooster, the presence of foam cells may be a secondary change accompanying the accumulation of lipid.

The essential features of human arteriosclerosis are found in the disease of the chicken. Both are primarily diseases of the intima rather than of the media, characterized by fibrous thickening with narrowing of the lumen. In both there occur atheromatous changes with mucoid degeneration and deposition of cholesterol and calcium within the thickened intima. There are, however, a number of species peculiarities requiring explanation. On the one hand, the longitudinal fibrous ridge which develops with such regularity in the abdominal aorta of the chicken has no counterpart in the human disease. On the other hand, the intimal ulceration and thrombosis observed in man do not occur in the chicken. No gross calcific plaques are found in the chicken but only microscopic calcific granules.

## SUMMARY AND CONCLUSIONS

Spontaneous arteriosclerosis of the aorta develops in 45 per cent of commercial roosters and hens over 1 year of age. The incidence of macroscopic lesions is the same in both sexes. Hens alone show fatty lesions of the intima of the ascending aorta and arch, while both roosters and hens commonly have intimal lesions of the abdominal aorta.

Arteriosclerosis in the chicken resembles human arteriosclerosis. Arteriosclerosis occurring spontaneously resembles that produced by cholesterol feeding.

The chicken is a suitable animal for the experimental production of arteriosclerosis if used before the age of 6 months, when spontaneous arteriosclerosis begins to occur. It is furthermore a suitable animal for studies on the prevention of arteriosclerosis because of the high incidence of the spontaneous disease and the early age at which it begins.

# INCIDENCE OF MAMMARY CARCINOMA IN MICE TREATED WITH ESTROGEN

## EFFECT OF THE AGE AT WHICH THE TREATMENT WITH ESTROGEN BEGINS

LEO LOEB, M.D., AND V. SUNTZEFF, M.D.
ST. LOUIS
AND
E. L. BURNS, M.D., AND I. R. SCHENKEN, M.D.
NEW ORLEANS

In earlier investigations [1] it was shown that there is a direct quantitative relationship between the duration of the action of endogenous ovarian hormones and the incidence of carcinoma of the mammary gland in mice; the longer the endogenous hormones were allowed to act, the greater the incidence of carcinoma. It was also shown that the latent period preceding the appearance of carcinoma of the mammary gland was in a similar way related to the duration of the action of the endogenous hormones; the longer the endogenous hormones were allowed to act, the shorter the latent period.[2]

In the investigations to be reported here we studied the effect of the age at which the administration of estrogen was begun on the incidence of carcinoma of the mammary gland in mice and on the length of the latent period which preceded its appearance. Two separate investigations were carried out. Experiment I was done by Loeb and Suntzeff in the laboratory of research pathology of the Washington University School of Medicine and experiment II by Burns and Schenken in the department of pathology and bacteriology of the Louisiana State University School of Medicine. Both of these experiments were carried out in pursuance of a plan to extend further previous investigations concerning the relations between age and the development of cancer. We studied the incidence of carcinoma, the preparatory growth and the processes of sensitization which may take place in the organ serving as a substratum for the action of the stimulating factors with special reference to the effect of variation in the age at which the stimulating factors began to act.

In both of these experiments estrogens were the stimulating factors, but stimulating factors of another type might have served for this purpose equally well. While in these two investigations the problem was the same, the methods employed were not the same. In experiment I estradiol benzoate was used; the quantity of this substance administered was adjusted to the weight of the mice at the various periods of life. Fully developed mice received 200 rat units weekly. The period during which injections were given lasted five months; after this period the mice were kept under observation without further administration of the estrogen. Male mice of two strains, C3H and D, were used, and four groups were distinguished according to the ages at which the administration of the estrogen was begun. Mammary gland tissue from each animal was studied microscopically.

In experiment II the stimulating factor used was estrone, which, because of its more rapid excretion, is probably less effective than estradiol benzoate. Each animal received 100 rat units weekly irrespective of weight. The subcutaneous injections of this preparation were continued throughout the life of the mice, whereas in experiment I the animals were killed with chloroform and the majority of them examined at an earlier period. In experiment II mice of strain C3H were used exclusively, but female as well as male mice were used. The injections of the estrogen were begun at three different periods of life. While paraffin sections of the mammary glands of many animals were examined, a more

From the Laboratory of Research Pathology, Washington University School of Medicine, St. Louis, and the Department of Pathology and Bacteriology, Louisiana State University School of Medicine, New Orleans.

This investigation was aided by a grant from the International Cancer Research Foundation. The experimental part of experiment I of this investigation was carried out by V. Suntzeff previous to June 30, 1941, when the aforementioned laboratory was discontinued; the microscopic investigation and the remaining work were carried out by Leo Loeb.

1. Loeb, L.: (*a*) Science **42**:912, 1915; (b) J. M. Research **40**:477, 1919; (*c*) Scient. Monthly **3**:209, 1916.

2. (*a*) Lathrop, A. E. C., and Loeb, L.: J. Cancer Research **1**:1, 1916. (*b*) Loeb, L.: ibid. **2**:135, 1917; (*c*) Compt. rend. Soc. de biol. **89**:307, 1923; (*d*) Presse méd. **31**:709, 1923; (*e*) J. Cancer Research **8**:274, 1924; (*f*) Acta, Union internat. contre cancer **2**:148, 1937; (*g*) footnote 1.

complete histologic study of these glands in the treated mice has not yet been undertaken. Notwithstanding these differences in the conditions under which the two investigations were carried out, the result seemed to be the same in both: The incidence of mammary carcinoma appeared to be influenced by the time at which the administration was begun.

## EXPERIMENT I

In order to avoid possible complications due to the action of endogenous estrogen, male mice were selected. These mice belonged to strains C3H and D in which the females have a high incidence of mammary carcinoma, which is caused by the combined action of endogenous ovarian hormones, the so-called milk factor and a genetic constitution which makes the mammary gland tissue responsive to the hormonal stimuli. All animals except those which died prematurely were given injections of estradiol benzoate for a period of five months.

The mice were divided into four groups according to the periods of life during which the injections of estrogen were begun. In group a the treatment began at the age of 2 to 2½ weeks, in group b at the age of 4 to 6 weeks, in group c at the age of 1¾ to 2¾ months and in group d at the age of 6 to 7 months. On the majority of the animals autopsies were done five to eight and one-half months after the end of the five month period of injections, but a certain number were examined at an earlier time. The quantity of estrogen injected subcutaneously was in each case adjusted to the weight of the animal; a mouse weighing 25 Gm. received 200 rat units of estrogen per week, and for young mice, weighing less, the amount administered was diminished proportionately to the difference in weight. Altogether, 44 mice of strain D and 34 mice of strain C3H were used in these experiments.

The discontinuation of our laboratory which was imminent at the conclusion of the experimental part of these investigations made an extension of the investigations, which would have comprised a larger number of animals, inadvisable; it also made impossible the observation of the animals over longer periods. Notwithstanding these restrictions, we believe that the results obtained strongly indicate that there is a relationship between the period of life when the administration of estrogen begins and the readiness with which mammary carcinoma develops subsequently. These conclusions were confirmed by the results obtained in experiment II, carried out by Burns and Schenken.

The effect of the administration of estradiol benzoate on the development of mammary carcinoma under the conditions of these experiments is seen in table 1. As regards both C3H and D mice, tumors originated in animals in which the injection of this estrogen began between the ages of 2 and 6 weeks (groups a and b); when it began between the ages of 1.75 and 7 months there were no tumors (groups c and d). The number of cancers which developed in groups a and b of strains C3H was greater than the number found in the corresponding groups of strain D. In strain C3H tumors formed in 30.7 per cent and 50.0 per cent of groups a and b, respectively (table 1). In strain D the percentages were 9.1 and 27.3 for groups a and b, respectively. In both strains combined, 21 per cent of the mice belonging to group a and 38.1 per cent of the mice belonging to group b had tumors. In none of the animals older than 6 weeks at the beginning of the treatment were tumors found during the period of observation.

In strain C3H the average interval between the last injection and death was about the same in group a and b, four and six-hundredths and four and five-hundredths months, respectively. The average age at death was about three weeks greater in group b than in group a in accordance with the fact that in group b the injections began two to three weeks later than in group a. In strain D a tumor developed in only 1 mouse in group a. In group b of this strain the average age at death was about fifteen days greater and the interval between the end of the injections and death was fifteen days longer than in strain C3H. The observed differences in tumor incidence and tumor age between strain C3H and strain D indicate that a more

TABLE 1.—*Effect of the Administration of Estrogen on the Mammary Glands of Mice in Strains C3H and D*

| | | Mice With Tumors | | | | Mice Without Tumors | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | Age at Start | Number | Per Cent | Average Age at Death, Mo. | Average Time Between Last Injection and Death, Mo. | Number | Per Cent | Average Age at Death, Mo. | Average Time Between Last Injection and Death, Mo. | Total Number of Mice |
| | | | | | Strain C3H | | | | | |
| a | 2 to 2.5 weeks | 4 | 30.7 | 9.44 | 4.06 | 9 | 69.3 | 10.19 | 5.7 | 13 |
| b | 4 to 6 weeks | 5 | 50.0 | 10.15 | 4.05 | 5 | 50.0 | 10.21 | 4.3 | 10 |
| c | 2 to 2.75 mo. | 0 | 0 | ..... | .... | 9 | 100.0 | 11.42 | 4.1 | 9 |
| d | 6.75 mo. | 0 | 0 | ..... | .... | 1 | 100.0 | 11.0 | 0 | 1 |
| | | | | | Strain D | | | | | |
| a | 2 weeks | 1 | 9.1 | 11.5 | 6.0 | 10 | 90.9 | 12.3 | 7.0 | 11 |
| b | 4 to 5 weeks | 3 | 27.3 | 10.44 | 4.04 | 8 | 72.7 | 12.15 | 8.15 | 11 |
| c | 1.75 to 2 mo. | 0 | 0 | ..... | .... | 14 | 100.0 | 13.91 | 6.98 | 14 |
| d | 6 to 7 mo. | 0 | 0 | ..... | .... | 8 | 100.0 | 14.6 | 3.25 | 8 |
| | | | | | Total of Strains C3H and D | | | | | |
| a | 2 to 2.5 weeks | 5 | 21.0 | 9.85 | 4.45 | 19 | 79.0 | 11.30 | 6.38 | 24 |
| b | 4 to 6 weeks | 8 | 38.1 | 10.61 | 4.38 | 13 | 61.9 | 11.40 | 6.67 | 21 |
| c | 1.75 to 2.75 mo. | 0 | 0 | ..... | .... | 23 | 100.0 | 12.93 | 5.85 | 23 |
| d | 6 to 7 mo. | 0 | 0 | ..... | .... | 9 | 100.0 | 14.2 | 3.25 | 9 |
| | | | | | Total of All Groups of Strains C3H and D | | | | | |
| a, b, c, d | 2 weeks to 7 mo. | 13 | 16.9 | 10.33 | 4.49 | 64 | 83.1 | 12.61 | 5.80 | 77 |

intense hormonal stimulation is necessary for the development of mammary carcinoma in strain D than in strain C3H.

In strains C3H and D the average age at death of the mice in which mammary carcinoma did not develop (table 1) was somewhat greater than that of the cancerous mice; likewise the interval between the last injection of estrogen and death was greater in the mice dying without tumors than in those dying with tumors. These findings applied equally to groups a and b. Of the corresponding figures that for strain D was greater than that for strain C3H in every case. If the mice of strain D had died as early as the mice of strain C3H the difference in tumor incidence between these two strains would have been still greater.

We compared the microscopic appearance of the mammary gland tissue of mice dying without tumors with that of the noncarcinomatous areas of the mammary gland tissue of mice dying with tumors. In order to make such a comparison possible we adopted the following grades: Grade 1 was given when no ducts were seen; grade 2, if a few ducts were seen; grade 3, if there were many ducts; grade 4, if in addition to the ducts a group or a few small groups of acini were observed, and grade 5, if one or several lobules of acini were present.

To be able to grade the growth and developmental processes in the mammary gland adequately it is advisable also to distinguish between mice which were examined within one month after cessation of the injections (class A) and those that were examined at a later time (class B). The distinction between these two classes should be made because there are indications that directly after the completion of the five month period of injections the mammary gland may show a farther going development than later, since during the rest period a partial regression may take place in the gland tissue which had previously grown as a result of the administration of estrogen.

Although the numbers of mice and mammary tumors shown in table 2 are small, there is a strong indication that the grades of mammary gland development outside the tumor areas were higher in strain C3H than in strain D, notwithstanding the greater average age of the mice in strain D. This means that the mammary glands developed more actively in the cancerous mice of strain C3H than in those of strain D, a conclusion which is in agreement with the greater incidence of cancers in strain C3H. There was no noticeable difference between the grades of group a and group b in strain C3H because the difference between the average ages of these two groups was slight. Likewise the average weights in groups a and b of strain C3H were about the same but were higher than those in strain D. Within class B of strains C3H and D there was no correlation between the weight of the individual cancerous mouse and the length of the interval between the end of treatment and the death of the animal (table 3).

Table 2 also shows that in the noncancerous mice of strain C3H the grades in class B reach a maximum in group b, in which there is also the maximum incidence of cancers. In groups a, b and c of strain C3H and in group b of strain D the grades are higher in class A than in class B, which is an indication that

TABLE 2.—*Grades Indicating the Development of the Noncarcinomatous Mammary Gland Tissues in Mice With and Without Mammary Tumors*

| | | Mice With Tumors | | | | | Mice Without Tumors | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Class | Number | Average Weight, Gm. | Grade * Range | Grade * Average | Average Interval Between Last Injection and Death, Mo. | Number | Average Weight, Gm. | Grade * Range | Grade * Average | Average Interval Between Last Injection and Death, Mo. | Total Number of Mice |
| | | | | | | Strain C3H | | | | | | |
| a | A | .. | .... | ... | .... | .... | 2 | 22.0 | 1-5 | 3.0 | .... | 2 |
| a | B | 4 | 24.4 | 1-5 | 3.75 | 4.06 | 7 | 25.0 | 1-5 | 2.6 | 5.7 | 7 |
| b | A | .. | .... | ... | .... | .... | 2 | 16.5 | 4-5 | 4.5 | .... | 2 |
| b | B | 5 | 24.7 | 1-5 | 3.80 | 4.05 | 3 | 21.8 | 2-5 | 3.4 | 4.3 | 8 |
| c | A | .. | .... | ... | .... | .... | 4 | 18.8 | 4-5 | 4.5 | .... | 4 |
| c | B | .. | .... | ... | .... | .... | 5 | 24.6 | 1-2.5 | 1.5 | 4.11 | 5 |
| d | A | .. | .... | ... | .... | .... | 1 | 16.0 | ... | 4.0 | .... | 1 |
| | | | | | | Strain D | | | | | | |
| a | B | 1 | 20.0 | ... | 2.5 | 6.0 | 10 | 23.45 | 1-4 | 2.4 | 7.0 | 11 |
| b | A | .. | .... | ... | .... | .... | 1 | 17.5 | ... | 4.0 | .... | 1 |
| b | B | 3 | 22.8 | 2-4 | 2.7 | 4.53 | 7 | 20.4 | 1-3 | 1.8 | 8.15 | 10 |
| c | B | .. | .... | ... | .... | .... | 14 | 19.3 | 1-4 | 2.4 | 6.98 | 14 |
| d | A | .. | .... | ... | .... | .... | 2 | 19.9 | 1-1 | 1.0 | .... | 2 |
| d | B | .. | .... | ... | .... | .... | 6 | 17.0 | 1-2 | 1.3 | 3.25 | 6 |

* Grade 1 was given when no ducts were seen; grade 2, when a few ducts were seen; grade 3, if there were many ducts; grade 4, if in addition to the ducts a group or a few small groups of acini were observed; grade 5, if one or several lobules of acini were present.

TABLE 3.—*Relationship of Interval Between Last Injection and Death to Weight of Mice with Mammary Carcinoma*

| Mouse No. | Group | Class | Interval Between Last Injection and Death, Mo. | Weight, Gm. |
|---|---|---|---|---|
| | | Strain C3H | | |
| 1 | a | B | 3.75 | 21.0 |
| 4 | a | B | 4.00 | 25.5 |
| 11 | a | B | 2.00 | 29.0 |
| 13 | a | B | 6.50 | 22.0 |
| 14 | b | B | 1.25 | 25.0 |
| 15 | b | B | 3.75 | 30.5 |
| 17 | b | B | 1.50 | 27.0 |
| 20 | b | B | 8.25 | 22.5 |
| 24 | b | B | 5.50 | 22.5 |
| | | Strain D | | |
| 1 | a | B | 6.00 | 20.0 |
| 16 | b | B | 2.75 | 27.5 |
| 20 | b | B | 5.00 | 20.0 |
| 22 | b | B | 6.00 | 21.0 |

at the conclusion of the period of injections or soon afterward the development of the mammary gland has reached a maximum and that subsequently regressive changes may set in.

In strain D there is no regularity as to the group in which the maximum development of the mammary gland is attained. As in the cancerous mice so also in the noncancerous mice there is a greater development of the mammary gland in strain C3H than in strain D. Moreover, the average grades of mammary gland development were higher in class B of cancerous mice than in class B of noncancerous mice.

## EXPERIMENT II

As mentioned earlier, this experiment differs from experiment I in various respects. Instead of the four groups in the first experiment, we separated the mice into only three groups, the first one of which corresponds approximately to the combined groups a and b of experiment I, while the second and third groups correspond to groups c and d, respectively, of experiment I. In experiment II 100 rat units of estrone was administered weekly to each mouse irrespective of weight, and the treatment was continued throughout the lifetime of the animals. Furthermore, females were used in addition to males, in this experiment, and the results obtained in them were compared with the incidence of mammary carcinoma and the age reached in nontreated female control mice. Mice belonging to strain C3H were used exclusively.

TABLE 4.—*Incidence of Mammary Carcinoma in Strain C₃H Mice When Estrone Was Started at Various Ages*

| Group | Age at Start | Males | | | Females | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Number | Number with Tumors | Per Cent with Tumors | Total Number | Number with Tumors | Per Cent with Tumors | Total Number | Total Number with Tumors | Per Cent with Tumors |
| I (corresponding to groups a and b)... | 10 days to 1 mo. | 13 | 5 | 38.4 | 11 | 9 | 81.8 | 24 | 14 | 58.3 |
| II (corresponding to group c)..... | 2 to 3 months | 16 | 0 | 0 | 11 | 3 | 27.2 | 27 | 3 | 11.1 |
| III (corresponding to group d)..... | 4 to 6 months | 23 | 1 | 4.3 | 18 | 12 | 66.6 | 41 | 13 | 31.7 |
| Totals..... | | 52 | 6 | 11.5 | 40 | 24 | 60.0 | 92 | 30 | 32.6 |
| Controls | | | | | | | | | | |
| Breeding..... | | No tumors observed | | | 97 | 76 | 78.3 | 97 | 76 | 78.3 |
| Nonbreeding..... | | No tumors observed | | | 38 | 6 | 15.7 | 38 | 6 | 15.7 |

The results obtained are shown in table 4. The incidence of mammary carcinoma in male mice was 38.4 per cent in group 1 (corresponding to groups a and b of the first experiment), 0 in group 2 (corresponding to group c) and 4.3 per cent in group 3 (corresponding to group d). These figures tend to confirm the conclusions arrived at in experiment I. The results were different in virgin female mice treated with estrone; here the corresponding percentages were 81.8, 27.2 and 66.6, respectively. There was therefore no definite relationship between the incidence of mammary carcinoma in female mice and the time of life at which the administration of the estrogen was begun. The average incidence (60.0 per cent) in the estrogen-treated virgin female mice was higher than that (15.7 per cent) in the control virgin female mice but it did not quite equal the incidence (78.3 per cent) noted in breeding female mice from strain C3H. The result obtained in the male mice in this experiment seemed thus to be in agreement with that obtained in experiment I. However, in view of the difference in the procedures used it seemed advisable to analyze further the figures of experiment II. There are two principal variable factors which complicate these results: (1) the time at which the treated animals died and (2) the length of the period during which the mice received injections of estrogen.

In table 5 the average age at death of the mice and the average length of the period during which estrone was administered, as well as the minimum and maximum limits of these two sets of data, are shown for the various groups of animals. Among the male mice the average ages at death are about the same in groups 1 and 3 in which the incidence of mammary carcinoma differs. It is therefore improbable that age is responsible for the difference in the results obtained in these two groups. However, the average length of the period of injections is greatest in the first group, in which the injections were begun at an earlier period of life. It is therefore possible that this factor complicates the results obtained as to the incidence of mammary carcinoma in the different groups of mice. On the other hand, in 2 mice of group 3 the average length of the period of injections almost equaled that of the cancerous mice of group 1, and in 10 mice it surpassed the length of this period; yet all of these mice remained free of mammary carcinoma. This makes it probable that the difference in the incidence of mammary carcinoma in the different groups cannot be attributed solely to the difference in the length of the period of injections but must be attributed largely to the difference in the age of the mice when the administration of the estrogen was begun.

Among the estrogen-treated female mice with mammary carcinoma there is no significant difference in the average age at death in various groups. As in the case of the males, the average length of the period of injections is greater in group 1 than in the other groups, and this difference in the average length of the period of injections is greater among the females than among the males; notwithstanding this fact, the difference in the incidence of mammary cancer in the females of groups 1 and 3 is relatively slight. As to the control mice, their average age at death was similar to that of the estrogen-treated females. This analysis makes it therefore probable that while the difference in the length of the period of administration of estrogen may be partly responsible for the results obtained, the principal factor is the difference in age at which the administration of estrogen was begun in the various groups of mice.

## COMMENT

Under the conditions present in both of these experiments a difference in the effectiveness of

the administration of estrogen was found to accord with the difference in the age of the mice at the time when administration of estrogen was begun. In the first experiment only injections beginning not later than the age of 5 or 6 weeks and continuing until the age of 5½ to 6¼ months led to the development of mammary carcinoma in male mice of strains C3H and D, which were both genetically predisposed to the formation of this type of tumor. In contrast to the positive results obtained under these conditions, injections of estrogen beginning at the age of more than 6 weeks and continuing up to the age of 6½ to 8 months or later did not lead to mammary carcinoma in these two strains within the time during which the animals were kept under observation. It was further noted that in mice in which the injections started at the age of 4 to 5 weeks the percentage of positive results was greater than in mice in which the injections began at the age of 2 weeks. Therefore the age period for the beginning of effective administration of estrogen ranged between the time preceding sexual maturity and the early period of sexual maturity. This period was followed by a sudden decline in the effectiveness of the injections of estrogen. The great difference in effectiveness is rather surprising in view of the fact that, although the treatment was started at different ages, it extended over much the same period of life in animals which became cancerous as in animals which did not. The observation that in principle the results obtained in this respect were the same in strains C3H and D indicates that the significance of this age factor is real. In both strains the peak of the tumor incidence was found at the same age. In accordance with the greater incidence of tumors in strain C3H it was also found that the development of the mammary gland tissue outside the tumor areas was greater in this strain than in strain D. Although the number of mice belonging to each group is not large, we believe it is sufficient to make this conclusion at least very probable.

Furthermore, the results obtained in experiment II agree with those in experiment I. However, in experiment II certain variable factors, which we have discussed, complicate the interpretation of the data. Notwithstanding these complications, it is probable that the difference in the incidence of cancers noted in mice belonging to group 1, in which the administration of estrogen began not later than the age of 1 month, and in those belonging to groups 2 and 3, in which the administration of estrogen began between the ages of 2 and 6 months, is to a large extent due to this age factor, although the variable factors mentioned may also have had some effect. The results obtained so far by Drs. Martin and Ruth Silberberg, who are extending these investigations in the department of pathology of New York University, point in the same direction.

More recently, in as yet unpublished experiments, Suntzeff and Cowdry found that in male mice of the New Buffalo strain whose skin was painted with methylcholanthrene cutaneous carcinoma developed much more frequently when painting was started at the age of 2½ to 3 months than when it was started at the age of 12 to 13 months. These results therefore agree in principle with our own, although the stimuli used and the periods of life at which the stimulation of the tissues was begun were not identical in these two investigations. The conclusion that the age at which the stimulus begins to act is of significance for cancerigenic effectiveness holds good, although in another strain (CBA) no

TABLE 5.—*Relation Between Age and Duration of Treatment, on One Hand, and Development of Mammary Carcinoma in Strain C3H Mice, on the Other*

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | Group 1 10 Days-1 Mo. | Group 2 2-3 Mo. | Group 3 4-6 Mo. | Group 1 10 Days-1 Mo. | Group 2 2-3 Mo. | Group 3 4-6 Mo. |
| Estrogen-treated mice with and without tumors | | | | | | |
| Average age at death, Mo. | 16.67 | 10.34 | 16.37 | 12.94 | 9.80 | 11.90 |
| Age range at death, Mo. | 6.10-19.94 | 7.37-13.64 | 10.03-21.20 | 10.0-16.07 | 6.24-13.70 | 9.54-14.94 |
| Average duration of treatment, Mo. | 15.64 | 7.70 | 11.10 | 12.40 | 7.40 | 6.57 |
| Range of duration of treatment, Mo. | 5.77-19.34 | 4.37-11.20 | 5.03-17.20 | 9.27-15.47 | 4.24-11.70 | 4.54-9.80 |
| Estrogen-treated mice with tumors | | | | | | |
| Average age at death, Mo. | 13.20 | ..... | 15.67 (1 mouse) | 12.60 | 13.07 | 12.80 |
| Age range at death, Mo. | 6.10-18.87 | ..... | ..... | 10.0-16.07 | 12.67-13.70 | 9.77-14.60 |
| Average duration of treatment, Mo. | 12.54 | ..... | 10.67 | 12.0 | 11.12 | 6.57 |
| Range of duration of treatment, Mo. | 5.77-18.34 | ..... | ..... | 9.27-14.0 | 10.67-11.70 | 4.77-9.47 |
| | Breeding Mice Without Tumors | Breeding Mice With Tumors | Virgin Mice Without Tumors | Virgin Mice With Tumors | | |
| Untreated female controls | | | | | | |
| Average age at death, Mo. | 12.28 | 12.67 | 11.87 | 10.10 | | |
| Age range at death, Mo. | 6.27-18.06 | 5.67-18.67 | 6.34-19.80 | 6.00-15.80 | | |

noticeable difference was found in the incidence of cutaneous cancers in similar age groups.

It may then be concluded that a relatively slight difference in the age when the administration of estrogen begins may cause a significant difference in the incidence of carcinomatous changes in the mammary glands of mice. It will therefore be necessary in experiments of this kind to consider the age of the animals at the time the treatment was begun in order to evaluate correctly the results obtained.

This age factor has differed in the experiments of different investigators. In our early experiments concerning the effect of endogenous ovarian hormones on the incidence of mammary carcinoma [2] the action of these hormones began at the period of sexual maturity. In the experiments of Lacassagne [3] on the effect of exogenous estrogen the administration of this substance was begun in mice soon after birth. In the majority of our later experiments with exogenous estrogen the injections began somewhat later in life [4] or, as in the experiments of Crossen and Loeb,[5] even in old age when the sexual activity of the mice was already declining. However, in none of the previous experiments on the origin of carcinoma of the mammary gland was the effectiveness of the administration of estrogen at different age levels directly compared.

While these experiments make it probable that a relatively slight difference in the age of the mice at the time when the hormone treatment is started influences the frequency of carcinomatous changes, they do not prove that administration of estrogen at later age periods is without effect on the production of carcinomatous changes in the mammary gland or, more generally expressed, that growth stimuli reaching the mammary gland in older mice are without any carcinogenic effect. The results obtained in experiment I which might suggest such a conclusion are limited in two respects: (1) The injections of estrogen extended only over a period of five months of the early life of these mice, and (2) the length of time during which these animals were kept under observation was relatively short; the oldest age reached by a mouse was 14.75 months and only eight and twenty-five hundredths months had elapsed in this instance since the last injection of estrogen; the other mice were examined at still somewhat earlier periods of life. It is likely that if the animals had been examined at a later date, the incidence of mammary carcinoma would have been greater. These two limiting factors were not present in experiment II. On the other hand, the estrogenic stimulating substance used in this experiment was less effective than the one used in experiment I. In experiment II a tumor did develop in group 3 in a mouse in which the administration of estrogen had begun at the age of 3 months; nevertheless, in this experiment the difference between the incidence in the various age groups was in principle the same as in experiment I. In both experiments I and II this age effect was found only in male mice. Among females, tumors were readily produced also in animals belonging to group 3, and no definite difference as to the effect of age at which administration of estrogen was begun was observed. Here administration of estrogen was about as effective in group 3 as in group 1; the difference in the incidence of mammary cancers observed in these two groups is probably not significant.

The conclusion that stimuli exerted at later periods of life may contribute to the production of mammary carcinoma is based on the fact that endogenous ovarian hormones are still active in this respect although their activity sets in only at the time of sexual maturity. This follows from the earlier experiments [6] in which it was shown that removal of the action of ovarian hormones at a period of life when the mice had passed the onset of sexual maturity was effective in diminishing the incidence of mammary carcinoma and in increasing the latent period. This observation is supported by the fact that exogenous estrogen may still be effective if its administration begins in fully adult mice, provided it is continued for a sufficiently long time; there are indications that even estrogen acting during the second year of life may intensify the development of cancer, as in the experiments of Crossen and Loeb.[5] Further support is provided by experiments in which it was shown that action of the ovarian hormones extending over a limited period and then discontinued may sensitize the mammary gland in such a way that from then on nonspecific stimuli may be able to exert cancerigenic effects on this organ and initiate mammary carcinoma.[7] In a similar way Leitch [8] and Bang [9] and others have shown that tar or

3. Lacassagne, A.: Compt. rend. Soc. de biol. **122**: 183, 1936.

4. Suntzeff, V.; Burns, F. L.; Moskop, M., and Loeb, L.: Am. J. Cancer **26**:761, 1936. Suntzeff, V.; Kirtz, M.; Blumenthal, H. T., and Loeb, L.: Cancer Research **1**:446, 1941.

5. Crossen, R. J., and Loeb, L.: Arch. Path. **37**:202, 1944.

6. Loeb.[2a, b] Lathrop and Loeb.[2a]

7. Loeb, L.: Virginia M. Semi-Monthly **13**:423, 1908; Arch. f. Entwcklngsmechn. **27**:73, 1909; **31**:456, 1911; Am. J. M. Sc. **159**:781, 1920; footnote 2 *f*.

8. Leitch, A.: Brit. M. J. **2**:1101, 1922.

9. Bang, F.: Compt. rend Soc. de biol. **87**:757, 1922.

carcinogenic hydrocarbons, too, acting over a restricted length of time may sensitize the tissue on which they act so that subsequently less specific or nonspecific stimuli can substitute for the specific ones and bring about a cancerous change in the affected tissues, although the nonspecific stimuli would not have been effective in nonsensitized tissues. Again, in this case, stimuli exert a cancerigenic influence on the tissues even of older animals. It has therefore been possible to distinguish in the cancerigenic action of stimuli two distinct processes: (1) actual, direct stimulation of growth and (2) sensitization consisting in a change of responsiveness of the tissue substratum to stimulation by hormones, by carcinogenic hydrocarbons and presumably by all types of stimuli provided they have acted with sufficient intensity and over a sufficient length of time. However, while this is merely a provisional distinction between two effects of stimulation which ultimately are probably the expression of the same underlying growth processes, the point in the growth curve of the tissue at which sensitization takes place is of real significance, and it is possible that younger tissues differ from older tissues not only in their intensity of growth, which is greater in younger tissues and gradually declines with advancing age, but also in the readiness with which the stage of sensitization is reached. It is further possible that the mutual antagonism of male and female sex hormones is involved in this effect of age on the origin of mammary carcinoma, in view of the fact that this age effect occurred only in male and not in female mice. However, the observation that in experiment I the difference in cancer incidence in groups b and c was great despite the facts that the administration of estrogen in group c began only a few weeks later than in group b and that then in both groups the age period over which the administration extended was largely the same, seems to make this interpretation of the significance of the age factor in male mice less probable, although it does not exclude it entirely. It is possible that in female mice the cancerigenic action of the endogenous hormones, which was continuous throughout a great part of the adult life of the mice, covers up the effect of the age at which the exogenous administration of estrogen began. Further investigations are needed to clear up these points.

As pointed out on previous occasions, two opposing sets of factors interact in determining the average incidence of cancer at the various age periods: 1. Younger tissues undergo more active growth than do older tissues, and each kind of tissue seems to have its own characteristic age curve. This relation between age and growth would favor the cancerous transformation of younger as compared with older tissues in animals, in accordance with the fact that cancer appears as the end stage of a connected and continuous series of gradually intensified growth processes. 2. On the other hand, the greater incidence of cancers in the tissues of older animals is favored by the fact that with increasing age an accumulation of growth stimuli as well as of processes of sensitization has had a better chance to take place than in the tissues of younger ones. Moreover, in the tissues of older animals processes of regeneration and repair occur less readily than in tissues of younger ones; in consequence of this condition there may be prolonged and abnormal growth processes; older tissues are less ready to adapt themselves to various types of injury or to strong stimulation in general, and this fact, too, may promote their ultimate cancerous transformation. These two sets of factors oppose each other, and in different cases one or the other may predominate. Under the conditions of these experiments the younger tissues responded to growth stimuli more readily with the development of carcinoma than the older tissues, and relatively small differences between the ages at which the stimulation set in led to great differences in the incidence of cancers in the mammary glands of the mice. Difference in the direct action of genetic factors and of the factor transmitted with the milk of the mother cannot be responsible for the difference in the results obtained, but indirectly cancerigenic genetic factors may be involved in the age effect inasmuch as they help to determine the different modes of reaction of tissues at different periods of life.

## SUMMARY

Two experiments were carried out to determine the effect of the age period at which the administration of estrogens began on the incidence of mammary carcnioma in mice. In the first experiment, in which also the growth processes in the mammary gland leading to the development of cancer were studied, male mice of strain C3H and strain D received subcutaneous injections of an estrogen, the amount of which was adjusted to the weight of the animal, a mouse weighing 25 Gm. receiving 200 rat units weekly. The administration of the estrogen was begun in various groups of mice at different age periods, which were as follows: (*a*) 2 weeks, (*b*) 4 to 6 weeks, (*c*) 1¾ to 2¾ months and (*d*) 6 to 7 months. Treatment was

continued for five months in each group. Mammary carcinoma developed only in groups a and b, the maximum in the incidence of these tumors being reached in group b. Mice of strains C3H and D behaved similarly in these respects, but in strain C3H the number of tumors in groups a and b was greater than the number in strain D. Likewise, the general development of the mammary gland tissues was, on the average, further advanced in mice which were bearers of mammary carcinoma than in mice not bearing tumors of this type, and it was somewhat further advanced in strain C3H than in strain D. In the second experiment similar results were obtained in strain C3H, although certain factors complicate the interpretation of the observations in this experiment. It was found that in female mice, in contrast to male mice, there was no significant difference in the incidence of mammary carcinoma in the different age groups. Differences in the growth energy or in the readiness of sensitization in young and old tissues or antagonism between male and female sex hormones is considered as possible causes of the observed age effects in the development of mammary carcinoma in mice.

Dr. Erwin Schwenk, of the Schering Corporation, Bloomfield, N. J., supplied the estradiol benzoate used in experiment I, and the G. W. Carnrick Company, Newark, N. J., supplied the estrone used in experiment II.

# Case Reports

## MELANOCARCINOMA OF THE CERVIX UTERI OR VAGINAL VAULT

CARL E. TAYLOR, M.D., AND HOWARD K. TUTTLE, M.D., ANCON, CANAL ZONE

Melanoma is among the commonest of tumors, but only rarely does it arise primarily in the female genital organs. Vulvar melanoma is a well recognized though infrequently encountered entity.[1]

There has been much discussion about the site of origin of melanoma of the ovary in the dozen or so cases which have been reported. It is agreed that in Amman's[2] case it arose in a dermoid cyst in an ovary. Miller[3] mentioned 3 cases, those of Andrews, Soubeyran and Rives, and Winternitz, which were reported as cases of primary ovarian melanoma. In his own and several other cases the growth was first thought to have been primary in the ovary but was subsequently shown to be metastatic from a pigmented nevus of the skin or a growth of the choroid which was diagnosed as melanoma. In the cases of Valdman-Gurevitch[4] and Dawson[5] metastases were found in the ovaries nine and thirteen years after the primary ocular tumor was removed by enucleation.

Dixon-Jones[6] presented a case in which melanoma seemed to have arisen in a fallopian tube, and Plate[7] surgically removed a large parametrial mass which was proved histologically to be melanoma. Cases of melanoma of the uterus have been described. Borst[2] is said to have collected 8 such cases, but he did not consider that in any of them the tumor was primary in the uterus. Similarly, Traina Rao[8] and Frank[9] concluded that the cases they reviewed were instances of metastasis. Balies[10] found branching cells laden with melanin in a uterine polyp.

Stefani,[11] Mulzer,[12] Boldt[13] and Smith and Leech[14] have presented well authenticated cases of melanoma of the vagina. In addition, Traina Rao[8] referred to 5 other cases and added 1 case of his own, making a total of 10 cases of vaginal melanoma.

We have been able to find no reports of melanoma of the cervix uteri. The 68 year old woman on whom Stefani[11] made an autopsy had an extensive vaginal tumor diagnosed as melanoma, which had invaded the cervix uteri. He discussed at some length the possibility that the tumor might have arisen in the cervix but finally decided that the vaginal vault was probably the primary site.

### REPORT OF A CASE

The patient was a white Spanish-born widow, who had lived in Panama since 1908. Her parents had died of old age; one brother was living and well, but five siblings had died of various noncontributory illnesses. Three adult children were living and well, while two others were dead. There was no history of cancer or other familial disease. Her past medical history was entirely without bearing on the case. In 1912 she had gone through an uneventful menopause.

She was first seen at Gorgas Hospital in 1930 at the age of 64 years. She complained of a bloody vaginal discharge and indefinite abdominal pains of one month's duration. Physical examination disclosed only a large sloughing tumor mass on the anterior lip of the cervix. The vaginal wall appeared normal. Oct. 17, 1930 an attempt was made to obtain a specimen for biopsy and to implant radium, with the patient under caudal epidural anesthesia induced with 90 cc. of 1 per cent procaine hydrochloride. Profuse hemorrhage necessitated the performance of vaginal hysterectomy with actual cautery. The tubes and ovaries were left in place. Convalescence was uneventful.

Dec. 27, 1934 the patient was readmitted, and two small dark tumors were excised from the vaginal wall, with the patient under nitrous oxide anesthesia. Aug. 23, 1935, while she was in the hospital because of dacryocystitis, a large black tarry lymph node was removed from the right inguinal region. On this admission she was found to be mildly diabetic. July 14, 1938 a black nodule which had appeared on the clitoris was excised. During hospitalization in February 1939 for the control of the diabetes, several pigmented areas were observed on both labia majora. In July 1941 she was again in the hospital for the control of diabetes, which continued to be mild. In addition to the pigmented areas on the vulva she had a zone of swelling and shallow ulceration around the urethral meatus. Jan. 17, 1942 she was admitted, complaining of urinary urgency, black urine, pruritus, pains in the right leg and vaginal bleeding. Inoperable melanocarcinoma of the vagina was present, and the labia majora were discolored purplish black.

From Gorgas Hospital, Ancon, Canal Zone.

1. Goforth, J. L.: Surg., Gynec. & Obst. **43**:322, 1926. Nucci, R. C.: Am. J. Obst. & Gynec. **36**:512, 1938. Johnson, W. O.: ibid. **37**:310, 1939.
2. Cited by Miller.[3]
3. Miller, J. R.: New England J. Med. **199**:830, 1928.
4. Valdman-Gurevitch, Z. I.: Klin. j. Saratov. Univ. **6**:121, 1928.
5. Dawson, H. G. W.: Brit. M. J. **2**:757, 1922.
6. Dixon-Jones, cited by Traina Rao.[8]
7. Plate, W. P.: Zentralbl. f. Gynäk. **63**:1194, 1939.
8. Traina Rao, G.: Riv. ital. di ginec. **17**:261, 1934.
9. Frank, R. T.: Gynecological and Obstetrical Pathology, New York, D. Appleton and Company, 1922, p. 253.
10. Balies: Ann. d'anat. path. **4**:373, 1927; cited by Stout, A. P.: Human Cancer, Philadelphia, Lea & Febiger, 1932, p. 341.
11. Stefani, A.: Tumori **9**:440, 1923.
12. Mulzer, A.: Arch. f. Gynäk. **130**:342, 1927.
13. Boldt, H. J.: Tr. New York Obst. Soc., 1906-1907, p. 153.
14. Smith, L. W., and Leech, J. V.: S. Clin. North America **8**:169, 1928.

There were three subsequent admissions for the same complaints during the next year. The patient's general condition deteriorated rapidly, while her tumor spread locally. She died April 30, 1943, at the age of 79 years.

*Biopsies.*—Cervix and uterus (1930): The fundus was small, atrophic and free from gross lesions. The surface and the margins of the cervix had been cauterized; on sectioning, several areas appeared slightly darker and firmer than the surrounding tissue.

Tumors from vaginal wall (1934): The two fragments of tissue measured 2 by 1 by 1 cm. and 1 by 1 by 0.5 cm. Each was composed principally of a nodule of black homogeneous material.

Right inguinal lymph gland (1935): The specimen was an oval lymph node, measuring 4.2 by 2.8 cm., which contained succulent brownish black material resembling tar.

Tumor of clitoris (1938): The specimen was grayish brown and measured 3.5 by 1.5 by 0.5 cm. In the center was a black nodular area.

*Autopsy* (1943).—Except for ascariasis and various degenerative manifestations, including generalized atherosclerosis, slight encephalomalacia and senile keratosis of the hands, the significant observations were limited to the tumor metastases. Careful examination of the skin revealed no tumor nodules.

The vaginal wall and the vulva had been replaced by an extensive necrotic and sloughing tumor composed of irregular, protuberant and superficially ulcerating black or reddish black tumor masses ranging up to 3 cm. in diameter; between them the residual skin and the mucous membrane appeared dark. Around the periphery of the vulva there were tiny seedlike, black nodules infiltrating the skin. The uterus was absent, the roof of the vagina being composed of an extremely thin layer of edematous connective tissue which had undergone extensive neoplastic infiltration. Several intestinal loops were bound to the pelvic floor by adhesions. Both the tubes and the ovaries were atrophic and free from neoplastic invasion. The tumor had invaded the floor of the bladder and infiltrated the urethral wall. There was no evidence of urethral obstruction, and the meatus was patulous. Metastases were found in the lungs, the myocardium, the diaphragm, the liver, the adrenal glands and the inguinal and pelvic lymph nodes. These metastatic nodules varied greatly in size and ranged from white through various shades of yellow and gray to coal black. Most of them were firm, although a few were soft and mushy.

*Microscopic Examination.*—In microscopic appearance the tumors removed surgically in 1930, 1934, 1935 and 1938 and those found at autopsy were essentially similar. There was some variation between nodules, two fairly well defined histologic types being recognized. The dark tumor which had deeply infiltrated the tissues of the cervix and the black metastatic nodules all contained abundant melanotic pigment. Their cellular pattern was moderately anaplastic, most of the cells having a spindle shape. The nuclei were large and hyperchromatic, and mitotic figures were rare. The cytoplasm was scanty and basophilic, and some of the cells contained many small granules of melanin. The pale metastatic nodules were relatively amelanotic, only a few cells containing tiny granules of pigment. The cellular pattern showed more anaplasia, most of the cells being large and having a polyhedral shape. The nuclei were large and vesicular, and they contained prominent nucleoli. Mitotic figures, including atypical forms, were more numerous than in the darker tumors. The cytoplasm was more abundant and showed fine basophilic stippling. Between these two histologic types there were the usual intermediate forms. All of the tumors were relatively vascular, some of the vessels having poorly defined walls. The stroma was scanty and appeared to be concentrated principally around the blood vessels. In both the lung and the heart there were distinct emboli of tumor cells lying free in the lumens of vessels some distance from tumor nodules. In a few of the metastases rare giant cells were seen, which appeared to have been formed by the fusion of several large polyhedral cells.

## COMMENT

Melanoma may metastasize so widely from a relatively insignificant primary lesion that it is usually difficult to rule out this possibility when a tumor of this type is found in a rare location. The most common primary sites are the skin and the retina. The skin of this patient was carefully examined, and no lesions were found. Permission for enucleation was not obtained, but the eyes were examined antemortem, and there were no visual disturbances during the thirteen year course of the disease. It would be most unusual for an ocular tumor to metastasize and recur so consistently in the uterine cervix and the vagina. It is felt, therefore, that there can be no reasonable doubt that this tumor arose on the anterior lip of the cervix or possibly in the vaginal vault immediately adjacent to the cervix so that the tumor could present at the cervix.

Masson's [15] theory of the neuroectodermal origin of melanocarcinoma has been widely accepted and confirmed.[16] Hirsch and Martin [17] have recently demonstrated sensory endings resembling Vater-Pacinian bodies in the myometrium and cervix. It is possible that the tumor arose from one of these bodies.

## SUMMARY

A patient with melanocarcinoma arising either in the cervix uteri or in the adjacent vaginal vault had a postoperative survival period of thirteen years, with multiple local recurrences, and finally died with general metastases. In a fairly comprehensive review of the literature no other cases of melanoma occurring primarily in this location were found.

15. Masson, P.: Ann. d'anat. path. **3**:417, 1926.
16. Foot, N. C.: Am. J. Path. **8**:309, 1932.
17. Hirsch, E., and Martin, M.: Surg., Gynec. & Obst. **76**:697, 1943.

# Notes and News

**Society News.**—The Biological Photographic Association will hold its fourteenth annual meeting on September 8 and 9 next in Binghamton, N. Y. For details address the secretary of the association, McGee Hospital, Pittsburgh.

S. P. Reimann is the new president-elect of the American Society of Clinical Pathologists; Alfred S. Giordano, South Bend, Ind., is the secretary-treasurer.

The new secretary-treasurer of the Society of American Bacteriologists is L. W. Parr, George Washington University Medical School, 1335 H Street, Washington 5, D. C.

The first lecture of the Institute of Medicine of Chicago in memory of Richard H. Jaffé, pathologist at Cook County Hospital, who died in 1937, was delivered on June 23 by William F. Petersen, who spoke on "Organic Variability in Heart Disease."

**Awards.**—The Ward Burdick Medal for 1944 of the American Society of Clinical Pathologists was awarded to Frank W. Hartman for his work on experimental hepatic necrosis.

The Squibb Award of the Association for the Study of Internal Secretions has been given to Edward A. Doisy, professor of biochemistry at St. Louis University for his "contributions to scientific knowledge, particularly in endocrinology."

**Deaths.**—Helion Povoa, professor of pathology at the University of Rio de Janeiro, Brazil, has died at the age of 45.

**Appointments.**—J. A. Ferrell, associate director of the International Health Division of the Rockefeller Foundation, has been appointed medical director of the John and Mary R. Markle Foundation for research in medical and physical sciences in the United States and Canada.

L. Pinheiro Guimares has been appointed professor of pathology in the School of Medical Sciences at Rio de Janeiro, Brazil.

In the University of Oregon Medical School Warren C. Hunter has been appointed director of the department of pathology, succeeding Frank R. Menne, who becomes pathologist to the St. Vincent's and Providence hospitals, Portland, Ore. Dr. Menne will continue as professor of pathology in the medical school.

Granville A. Bennett, professor and head of the department of pathology at Tulane University, New Orleans, has accepted appointment to a similar position in the University of Illinois College of Medicine, Chicago.

# Books Received

TROPICAL DISEASES. By Ernest Carroll Faust, Ph.D., professor of parasitology, department of tropical medicine, Tulane University of Louisiana, New Orleans. Pp. 49. Seattle: Metropolitan Press Printing Co., 1944.

This is the third series of Somner Memorial Lectures at the University of Oregon Medical School. The lectures deal with the following topics: old and new horizons of American tropical medicine; insects and their allies as causative agents and transmitters of disease; malaria; yellow fever, dengue and sandfly fever; amebiasis and related infections; filariasis. The presentation is of special interest and value at this time. The lecures are reprinted from *Northwest Medicine*. Free copies can be obtained from Dr. Frank R. Menne, University of Oregon Medical School, Portland, and from Dr. Ernest C. Faust, 1430 Tulane Avenue, New Orleans 13. The cost of mailing is 10 cents a copy.

THE ROCKEFELLER FOUNDATION: ANNUAL REPORT, 1943. Pp. 329, illustrated. New York: Rockefeller Foundation, 1944.

THE ELECTROCARDIOGRAM, ITS INTERPRETATION AND CLINICAL APPLICATION. By Louis H. Sigler, M.D., attending cardiologist and chief of cardiac clinics, Coney Island and Harbor Hospitals, Brooklyn. Pp. 403, with 203 illustrations. Price $7.50. New York: Grune & Stratton, Inc., 1944.

THE INTERNATIONAL CANCER RESEARCH FOUNDATION: REPORT OF ACTIVITIES DURING 1943. Pp. 108. Philadelphia: The International Cancer Research Foundation, 1944.

HYPERTENSION AND HYPERTENSIVE DISEASE. By William Goldring, M.D., associate professor of medicine, New York University College of Medicine; chief, Nephritis and Hypertension Clinic, New York University Clinic; and Herbert Chasis, M.D., assistant professor of medicine, New York University College of Medicine; associate chief, Nephritis and Hypertension Clinic, New York University Clinic. Pp. 253, with 53 illustrations. Price $3.50. New York: The Commonwealth Fund, 1944.

## CORRECTION

In the article by Maurice N. Richter entitled "Automatic Staining of Routine Tissue Sections with Hematoxylin and Eosin," in the May issue (ARCH. PATH. **37**:338, 1944) "10 cc." in the fourth line of the first column on page 339 should read "10 Gm."

THE ARCHIVES OF PATHOLOGY is published by the American Medical Association as a medium to advance pathology in the United States and to promote research and observation in this field.

Manuscripts for publication, books for review and correspondence relating to contributions should be sent to Dr. Ludvig Hektoen, Chief Editor, 21 West Elm Street, Chicago 10, or any other member of the Editorial Board. Communications regarding subscriptions, etc., should be addressed, ARCHIVES OF PATHOLOGY, American Medical Association, 535 North Dearborn Street, Chicago 10.

Articles are accepted for publication on condition that they are contributed solely to the ARCHIVES OF PATHOLOGY. Manuscripts must be typewritten, preferably double spaced, and the original copy should be submitted. Zinc etchings and halftones will be made by the Association when the original illustrations warrant reproduction and when their number is not considered excessive.

Footnotes and bibliographies (the latter are used only in exhaustive reviews of the literature) should conform to the style of the *Quarterly Cumulative Index Medicus*, and should be in the order given: name of author and name of periodical, with volume, page and year (titles are not used in this journal).

Matter appearing in the ARCHIVES OF PATHOLOGY is covered by copyright, but as a rule no objection will be made to its reproduction in a reputable medical journal provided credit is given. However, the reproduction for commercial purposes of articles appearing in the ARCHIVES OF PATHOLOGY or in any of the other publications issued by the Association will not be permitted.

The ARCHIVES OF PATHOLOGY is published monthly. The annual subscription price (for two volumes) is as follows: Domestic, $6.00; Canadian, $6.40; foreign, $7.00 (including postage). Single copies, 75 cents, postpaid, except special issues.

Checks, money orders and drafts should be made payable to the American Medical Association.

---

## OTHER PERIODICAL PUBLICATIONS
### of the American Medical Association

**THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION**—Weekly. Covers all the medical sciences and matters of general medical interest. Illustrated. Annual subscription price (three volumes): domestic, $8.00; Canadian, $9.00; foreign, $12.00. Single copies, 50 cents.

**ARCHIVES OF INTERNAL MEDICINE**—Monthly. Devoted to the publication of advanced original clinical and laboratory investigations in internal medicine. Illustrated. Annual subscription price (two volumes): domestic, $5.00; Canadian, $5.60; foreign, $6.00. Single copies, 75 cents.

**AMERICAN JOURNAL OF DISEASES OF CHILDREN**—Monthly. Presents pediatrics as a medical science and as a social problem. Includes carefully prepared reviews, based on recent pediatric literature, abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): domestic, $6.00; Canadian, $6.60; foreign, $8.00. Single copies, 75 cents.

**ARCHIVES OF NEUROLOGY AND PSYCHIATRY**—Monthly. A medium for the presentation of original articles on nervous and mental diseases, with abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): domestic, $6.00; Canadian, $6.60; foreign, $8.00. Single copies, 75 cents.

**ARCHIVES OF DERMATOLOGY AND SYPHILOLOGY**—Monthly. Devoted to advancing the knowledge of and progress in cutaneous diseases and syphilis. Publishes original contributions and abstracts of literature on these two subjects, transactions of the important dermatologic societies, book reviews, etc. Illustrated. Annual subscription price (two volumes): domestic, $6.00; Canadian, $6.40; foreign, $8.00. Single copies, 75 cents.

**ARCHIVES OF SURGERY**—Monthly. Devoted largely to the investigative and clinical phases of surgery, with monthly reviews on orthopedic and urologic surgery. Well illustrated. Annual subscription price (two volumes): domestic, $8.00; Canadian, $8.40; foreign, $9.00. Single copies, $5 each, except special issues.

**ARCHIVES OF OTOLARYNGOLOGY**—Monthly. A medium for the presentation of original articles on diseases of the ear, nose and throat, with abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): domestic, $6.00; Canadian, $6.40; foreign, $7.00. Single copies, 75 cents.

**ARCHIVES OF OPHTHALMOLOGY**—Monthly. Includes original articles on diseases of the eye, abstracts from foreign and domestic literature, book reviews, transactions of special societies, etc. Illustrated. Annual subscription price (two volumes): domestic, $8.00; Canadian, $8.40; foreign, $9.00. Single copies, $5 each.

**WAR MEDICINE**—Monthly. Devoted to material on preparedness and scientific advancement in relation to military, naval, public health and allied services. Annual subscription price (two volumes): domestic, $6.00; Canadian, $6.40; foreign, $7.00. Single copies, 75 cents.

**QUARTERLY CUMULATIVE INDEX MEDICUS**—Quarterly. A complete subject and author index to the worth while current medical literature of the world. Issued four times a year. Second year's issue volumes bound for permanent reference. Subscription price, calendar year: domestic, $13.00; Canadian, $14.00; foreign, $14.00.

---

AMERICAN MEDICAL ASSOCIATION
535 North Dearborn Street CHICAGO 10

# CONTENTS


Page

Infantile Toxoplasmosis, with a Report of Three New Cases, Including Two in Which the Patients Were Identical Twins. Wolf W. Zuelzer, M.D., Detroit ..... 1

Parathyropituitary Syndrome (Pituitary Dysfunction and "Primary" Hyperparathyroidism). Robert M. Perlman, M.D., Bethesda, Md. ..... 20

Protein as Oxidase. Martin H. Fischer, M.D., Sc.D., and Werner J. Suer, M.S., Cincinnati ..... 28

Morphologic and Histochemical Study of the Effect of Scurvy on Tuberculosis in Guinea Pigs, and of the Origin, Amount and Distribution of Alkaline Phosphatase in the Foci of Caseous Necrosis. William O. Russell, M.D., and James Allan Read, M.D., St. Louis, and Lieutenant Ernest T. Rouse, Medical Corps, Army of the United States ..... 31

A Histochemical Study of the Effect of Scurvy on the Activity of Alkaline Phosphatase in the Kidneys of Guinea Pigs. William O. Russell, M.D., St. Louis; Lieutenant Ernest T. Rouse, Medical Corps, Army of the United States, and James Allan Read, M.D., St. Louis ..... 40

Spontaneous Arteriosclerosis in Chickens. D. V. Dauber, M.D., Chicago ..... 46

Incidence of Mammary Carcinoma in Mice Treated with Estrogen: Effect of the Age at Which the Treatment with Estrogen Begins. Leo Loeb, M.D., and V. Suntzeff, M.D., St. Louis, and E. L. Burns, M.D., and J. R. Schenken, M.D., New Orleans ..... 52

Case Reports:

Melanocarcinoma of the Cervix Uteri or Vaginal Vault. Carl E. Taylor, M.D., and Howard K. Tuttle, M.D., Ancon, Canal Zone ..... 60

Notes and News ..... 62

Books Received ..... 63